A study of the damage of articular cartilage caused by crystals

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A STUDY OF THE DAMAGE OF ARTICULAR CARTILAGE CAUSED BY CRYSTALS

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CONTENTS

SUMMARY 1

1. INTRODUCTION 3

2. ARTICULAR CARTILAGE: ITS STRUCTURE AND MECHANICAL PROPERTIES, DAMAGE AND DISEASES 5

2.1 THE STRUCTURE AND COMPOSITION OF ARTICULAR CARTILAGE 5

2.1.1 Collagen 6
2.1.2 Proteoglycans 8
2.1.3 Water 10
2.1.4 Summary 10

2.2 THE MECHANICAL PROPERTIES OF ARTICULAR CARTILAGE 11

2.2.1 The Viscoelastic Nature of Articular Cartilage 11
2.2.2 The Permeability of Articular Cartilage 13
2.2.3 Behaviour of Articular Cartilage in Tension 13
2.2.4 Behaviour of Articular Cartilage in Pure Shear 14

2.3 LUBRICATION OF ARTICULAR CARTILAGE 15

2.3.1 Historical Background to the Lubrication of Synovial Joints 15
2.3.2 Lubrication Mechanisms Operative Under Varying Load Conditions 17

2.4 DAMAGE OF ARTICULAR CARTILAGE AND OSTEOARTHRITIS 18

2.4.1 Fragmentation of the Collagen Network 19
2.4.2 Loss of Proteoglycans and Increase in Water Content 21
2.4.3 Other Mechanisms of Cartilage Damage 21

2.4.4 Biochemical Factors 24

2.5 CRYSTAL DEPOSITION DISEASES 25

2.5.1 Acute Inflammatory Diseases 27

2.5.2 Chronic Destructive Joint Disease 28

2.5.3 The Crystals 31

2.6 SUMMARY 33

FIGURES 34

3. THE FRICTIONAL RESPONSE OF ARTICULAR CARTILAGE 40

3.1 INTRODUCTION 40

3.2 LITERATURE REVIEW 41

3.2.1 Values Previously Recorded for the Coefficient of Friction of Articular Cartilage 41

3.2.2 Experimental Details for Measuring the Coefficient of Friction of Articular Cartilage 42

3.3 THE FRICTION APPARATUS 46

3.3.1 The Original Design of Friction Apparatus 46

3.3.2 Modifications to the Friction Apparatus 47

3.4 MATERIALS 48

3.4.1 Source of Cartilage 48

3.4.2 Storage of the Specimens 49

3.4.3 Synthetic Crystals 49

3.5 EXPERIMENTAL METHOD 51

3.5.1 Location of the Normal Position 51

3.5.2 Replacement of the Hemispherical Glass Indentor 52

3.5.3 Quantification of the Frictional Response of Crystals on the Cartilage Surface 53

- Crystal Deposition onto the Cartilage Surface 54

3.5.4 Grading the Condition of the Bone-Cartilage Interface 56

3.6 RESULTS AND DISCUSSION 57

3.6.1 Ploughing Effects Caused By The Small Glass Indentor 57

3.6.2 Results Obtained Using a Flat Glass Plate 60

3.6.3 Effect of Motor Speed on the Coefficient of Friction Measured With a Flat Glass Plate 64

3.6.4 Use of a Large Cylindrical Indentor 66

3.6.5 Effect of Curvature of the Specimen and Starting Position on the Coefficient of Friction 67

3.6.6 Quantitative Analysis of the Frictional Response of Crystals on the Surface of Articular Cartilage 68

3.6.7 Relationship Between the Bone-Cartilage Interface, the Crystal Density within the Cartilage, and the Fibrillation of the Cartilage 71

3.7 CONCLUSIONS AND FURTHER DEVELOPMENT 73

FIGURES 76

4. THE WEAR RESPONSE OF ARTICULAR CARTILAGE 83

4.1 INTRODUCTION 83

4.2 LITERATURE REVIEW 86

4.3 THE WEAR APPARATUS 95

4.3.1 The Apparatus Used to Measure the Wear and Creep of Articular Cartilage 96

4.4 MATERIALS 97

4.4.1 Source of Cartilage Specimens 97

4.4.2 Storage of the Specimens 99

4.4.3 Preparation of Synthetic Crystals 100

4.4.4 Characterisation of the Synthetic Crystals 101

4.5 EXPERIMENTAL METHOD 103

4.5.1 Method Used to Measure the Wear of Articular Cartilage 104

4.5.2	Calibration of the LVDT	108
4.5.3	Method Used to Measure the Creep Deformation of Articular Cartilage	109
4.5.4	Estimation of Wear by Quantification of Sulphated Glycosaminoglycans	109
4.5.5	Determination of a GAG Masking Effect by the Presence of Crystals in Solution	111
4.5.6	Estimation of Wear by Determination of Bound Sulphate by Ion Chromatography	112
4.5.7	Preparation of Cartilage Wear Samples for Scanning Electron Microscopy	114
4.5.8	Preparation of Cartilage Samples for Transmission Electron Microscopy	115
4.6 RESULTS AND DISCUSSION		116
4.6.1	Control Tests to Ascertain Whether Proteoglycans or GAGs Were Leached From the Cartilage When Under Load	116
4.6.2	Pilot Wear Experiments With Crystals in the Lubricant and the Subsequent Determination of Masking of the Sulphated GAGs by the Crystals	117
4.6.3	Repeatability of the Spectrophotometric Assay	123
4.6.4	Increase in GAG Concentration With Time When Measured With the Spectrophotometric Assay	123
4.6.5	Correlation Between the Spectrophotometric Assay and the Ion Chromatography	125
4.6.6	Repeatability of the Ion Chromatography	126
4.6.7	The Wear of Articular Cartilage Over a Range of Normal Loads	127
	- Load Dependence of Wear	128
	- Dependence of Wear on Contact Stress	129
	- Errors Associated With Measuring the Contact Area	130
	- Summary	132

4.6.8	The Wear of Articular Cartilage With and Without Crystals Present in the Lubricant	133
4.6.9	Measurement of the Vertical Displacement of the Cartilage During the Wear Test	135
	- Determination of the Creep Component	136
	- Errors Associated With the LVDT	137
	- LVDT Measurements With and Without Crystals Present in the Lubricant	138
4.6.10	Comparison of Theoretical Predictions of the Thickness of Cartilage Removed With Experimental Measurements	138
4.6.11	Comparison of the Mean Contact Areas Before and After the Wear Test	141
	- Summary	143
4.6.12	The Wear of Articular Cartilage With Time Measured With the LVDT and Ion Chromatography	144
4.6.13	Wear of Articular Cartilage Scratched In Vivo	148
4.6.14	Creep of Normal Articular Cartilage and Cartilage Scratched In Vivo	153
4.6.15	Comparison of the Creep and Consecutive Wear Response of Normal Articular Cartilage	155
4.6.16	Transmission Electron Microscopy of Transverse Sections of Equine Articular Cartilage	164
4.6.17	Optical and Scanning Electron Microscopy of Cartilage Surfaces Worn With Crystals Present in the Lubricant	165
	- The Raised Blisters	166
	- Parallel Scratch Marks	167
	- Crystal Debris	170
	- Rucking of the Cartilage Surface	171
	- Damage of the Cartilage Outside the Contact Area	173
	- Summary	177

4.6.18 Optical and Scanning Electron Microscopy of Cartilage Worn Under High and Low Nominal Contact Stresses 178

4.6.19 Optical and Scanning Electron Microscopy of Cartilage Scratched In Vivo 180

4.7 CONCLUSIONS 182

FIGURES 191

5. CRYSTAL AGGREGATES IN ARTICULAR CARTILAGE 233

5.1 INTRODUCTION 233

5.2 LITERATURE REVIEW 234

5.3 MATERIALS AND METHODS 238

5.4 RESULTS AND DISCUSSION 240

5.4.1 Monosodium Urate Crystal Aggregate in Articular Cartilage 241

5.4.2 Calcium Pyrophosphate Dihydrate Crystal Aggregates in Articular Cartilage 242

5.4.3 The Association of the Crystal Aggregates With Cartilage Damage 244

5.4.4 Characterisation of Crystals in Equine Articular Cartilage 247

5.5 CONCLUSIONS 248

FIGURES 250

6. CONCLUSIONS AND FURTHER WORK 255

6.1 The Frictional Response of Articular Cartilage With and Without Crystals 255

6.2 The Wear Response of Articular Cartilage With and Without Crystals 256

6.3 The Wear Response of Articular Cartilage Scratched In Vivo 258

6.4 The Nature of the Relative Bearing Surfaces 259

6.5	The Association Between Crystal Aggregates and Cartilage Damage	259
6.6	Summary	260

ACKNOWLEDGEMENTS 261

APPENDIX 264

REFERENCES 275

SUMMARY

There has been much speculation about whether pathological crystal deposits in synovial fluid or articular cartilage of a synovial joint can cause mechanical damage of the tissue. The aims of this thesis were (i) to investigate whether crystals in a lubricant could cause damage to articular cartilage *in vitro*, and (ii) to investigate any association between crystal deposits within the cartilage and visible cartilage damage.

Investigations of the frictional response of articular cartilage with surface crystals were performed. Results indicated that the coefficient of friction varied with crystal type and was influenced by the crystal morphology.

Further study of the wear response of articular cartilage indicated that the wear of normal, initially undamaged articular cartilage was dependent on the load applied and the nominal contact stress generated. The presence of crystals in the lubricant significantly increased the wear of the cartilage. Scanning electron microscopy results suggested that the type of damage sustained by the cartilage was influenced by the crystal type.

Cartilage that had been damaged *in vivo* and exhibited parallel scratch marks generally showed no significant difference in its wear response compared to normal, undamaged cartilage. There was, therefore, no clear evidence to suggest that the wear response of this scratched cartilage had been compromised.

Scanning electron microscopy studies of transverse sections of human articular cartilage provided clear evidence that crystal deposits within the articular cartilage were often associated with cracks through the cartilage, but large isolated crystal aggregates were also observed deep within undamaged articular cartilage.

This study has provided evidence to support the hypothesis that crystals can cause mechanical damage of articular cartilage.

1. INTRODUCTION

Many arthritic diseases affect the synovial joints of the human body, the most common being osteoarthritis. This disease is a major cause of musculoskeletal pain and disability in the United Kingdom and is characterised by the degeneration of the articular cartilage. The principal sufferers are the elderly, (Sokoloff, 1969). Crystal deposition in the articular cartilage, or the synovial fluid, of a joint also occurs commonly in the elderly, (Dieppe & Calvert, 1983). There has been much debate about the relative association between the deposition of crystals and the disease of osteoarthritis (see section 2.5.2), in particular, with respect to the role that crystals may play in the damage and eventual destruction of the cartilage layer. Several authors have postulated that crystals present in the articular cartilage or the synovial fluid could cause mechanical damage to the tissue by altering its compliance or by surface abrasion, respectively, (Dieppe & Doherty, 1982, Dieppe & Calvert, 1983, and Dieppe & Watt, 1985). However, there has been no experimental work to investigate possible mechanisms by which crystals could mechanically damage the articular cartilage.

The aims of this study were: (i) to investigate whether crystals in a lubricant could cause damage to articular

cartilage *in vitro*, and (ii) to investigate any association between crystal deposits within the cartilage and overt cartilage damage.

Initially, an investigation of the frictional properties of articular cartilage with and without surface crystals was carried out. However, this work was dependent on the assumption that a high measured coefficient of friction implied a high potential for damage, or wear, of the cartilage. Such a relationship is unsubstantiated. Further work, therefore, concentrated upon obtaining a quantitative biochemical measure of the mechanical damage incurred by the articular cartilage *in vitro* with and without crystals present in a lubricant. Scanning electron microscopy was used to study the morphology and distribution of crystal aggregates within articular cartilage and their association with cartilage damage.

It was anticipated that this research would address the suggestion of Dieppe and his colleagues (Dieppe & Doherty, 1982, Dieppe & Calvert, 1983, and Dieppe & Watt, 1985), that damage of articular cartilage could be caused by crystals, by providing experimental evidence of potential mechanisms.

2. ARTICULAR CARTILAGE: ITS STRUCTURE AND MECHANICAL PROPERTIES, DAMAGE AND DISEASES

Articular cartilage is one of the basic components of a synovial joint (figure 2.1). There are three types of joint in the body: synarthrosis (non-moving) joints, amphiarthrosis (little-moving) joints, and diarthrosis (freely-moving) or synovial joints. Synovial joints basically consist of two bone ends, covered by articular cartilage and enclosed by a synovial membrane. Between the articular surfaces of a healthy joint there is a small volume of synovial fluid (typically 1ml). A synovial joint is stabilised mechanically by strategically placed ligaments.

2.1 THE STRUCTURE AND COMPOSITION OF ARTICULAR CARTILAGE

Normal, healthy articular cartilage has a smooth, glistening whitish surface. It is firm but feels slippery to the touch. If the articular surface is wiped dry and the cartilage deformed, fluid can be seen to weep from the surface. The thickness of articular cartilage varies from joint to joint, and from site to site in the same joint (Van Sickle & Kincaid, 1978), but normally ranges from 2-5mm in humans, less in other animals. It has two primary functions: to distribute joint loads over a wide area, thus decreasing the contact stresses, and to allow relative movement of

opposing joint surfaces with minimal friction and wear, (Mow *et al*, 1989).

Articular cartilage has no blood vessels, lymph channels or nerves and is, therefore, physiologically quite isolated. The bulk of the structure is made up of intercellular ground substance consisting of a swollen gel containing 10-30% collagen fibres and 3-10% proteoglycan molecules (PGs). The remaining 60-87% is water, inorganic salts and small amounts of other matrix proteins, glycoproteins and lipids, (Mow *et al*, 1989). The sparsely distributed cartilage cells, or chondrocytes, manufacture, secrete and maintain the organic component of the cartilage matrix. The collagen fibres and PGs, together with water, determine the biomechanical behaviour of articular cartilage.

2.1.1 Collagen

Collagen fibres consist of three procollagen polypeptide chains (α chains) coiled into left-handed helices, which are then further coiled into a right-handed triple helix. The tropocollagen molecules thus created align parallel to each other, overlapping by one quarter of their length, and polymerise into larger collagen fibres, with covalent cross-links adding to the fibres' high tensile strength. There are several different types of collagen. Type II and type IX are predominantly observed in articular cartilage. It has been suggested

(Muir, 1990) that the type IX collagen has a role in stabilising the fibrillar network, which mainly consists of type II collagen fibrils.

The collagen in articular cartilage is inhomogeneously distributed throughout the tissue thickness. A zonal arrangement has been generally accepted with sheets of fine, densely packed fibres randomly woven in planes parallel to the articular surface in the superficial zone, randomly orientated and homogeneously dispersed fibres in the mid-zone and radially orientated fibre bundles in the deep, or calcified, zone that form an interlocking system anchoring the cartilage to the underlying bone, (figure 2.2). Recent work by Clark (1990), however, indicated that fibres which originated in the radial (or deep) zone could be traced into the surface where they flattened and overlapped in a common direction. These tangential fibres were then covered by a dense, separate layer of small fibrils. The collagen content is highest at the surface and then relatively constant throughout the deeper zones (Lipshitz *et al*, 1975).

The most important mechanical properties of collagen fibres are their tensile stiffness and strength. The tensile stiffness of tendons (which are about 80% collagen by dry weight) is 1GPa with a tensile strength of 50MPa (Mow *et al*, 1989). However, collagen fibres are weak in compression owing to their high length-to-thickness ratio making it easy for them to buckle.

Articular cartilage is anisotropic and it is thought that this anisotropy is related to the varying orientation of the collagen fibre network, variations in collagen fibre cross-link density and variations in collagen-PG interactions. In tension, this can be illustrated by the split line pattern (elongated fissures produced by piercing the articular surface with a small round awl), (see figure 2.3).

2.1.2 Proteoglycans

Proteoglycans (PGs) exist either as monomers or large aggregates (figure 2.4). The bottle-brush-like PG monomer consists of a protein core to which glycosaminoglycan (GAG) chains are covalently attached. The sulphated GAGs present in articular cartilage are chondroitin (CS) and keratan sulphate (KS) which are distributed heterogeneously along the protein core. In cartilage most PG monomers form aggregates by noncovalently attaching to a central hyaluronic acid core stabilised by link proteins. It is now generally accepted (Mow *et al*, 1989) that PG aggregation promotes immobilisation of the PGs within the collagen meshwork, adding structural rigidity to the extracellular matrix.

A high swelling pressure within cartilage and a low hydraulic permeability leads the PG gel to form the compression-resisting element in cartilage. The high swelling pressure is caused by the flexible, hydrophilic

nature of the GAG chains and their high concentration of negatively fixed charges, (Maroudas, 1979). These result from dissociation of the sulphate and carboxyl groups on the GAGs in solution at physiological pH. The resulting strong repulsive charge forces extend and stiffen the macromolecules in the interfibrillar space formed by the collagen network. Mobile cations in the solution (i.e. the synovial fluid) are positively attracted to the fixed anionic groups on the GAGs in the cartilage and because of this the distribution of mobile cations is more than in the synovial fluid. The component of the osmotic swelling pressure due to this excess of ions is referred to as the Donnan osmotic swelling pressure, (Maroudas, 1979, and Mow *et al*, 1989). The ionic contribution to the osmotic swelling pressure is approximately 0.17MPa, whereas the total swelling pressure is approximately 0.2MPa, (Maroudas, 1979). The Donnan osmotic swelling pressure, therefore, constitutes the main contribution. The total swelling pressure is resisted and balanced by tension developed in the collagen network, which confines the PGs to only 20% of their free solution domain (Mow *et al*, 1989).

The PGs also interact with the collagen fibres creating links across distances too great for collagen cross-links to develop (Mow *et al*, 1989). PGs are also thought to play an important role in maintaining the ordered structure and hence the associated mechanical properties of the collagen fibres. The elastic modulus of the

collagen-PG matrix is approximately 0.78MPa (Mow *et al*, 1989). The ability of the PGs to resist compression, therefore, arises from two sources: the osmotic swelling pressure mainly associated with the tightly packed negatively fixed charges on the GAGs and the bulk compressive stiffness of the PG aggregates entangled in the collagen network (Mow *et al*, 1989).

2.1.3 Water

Water is the most abundant component of articular cartilage and is essential to the health of this avascular tissue permitting the diffusion of gases, nutrients and waste products between the chondrocytes and the synovial fluid. Most of the water in cartilage is extracellular (Sokoloff, 1969) and is free to move through the tissue when a load is applied (Maroudas, 1979). This movement is important in controlling the mechanical behaviour of cartilage. The time-dependent response of cartilage to an applied force is influenced strongly by the movement of this interstitial fluid, (Kempson, 1979).

2.1.4 Summary

The structural macromolecules of collagen and PG interact to form a porous, composite, fibre reinforced matrix possessing all the essential mechanical characteristics of a solid that is swollen with water.

When articular cartilage is subjected to external loads, the collagen-PG solid matrix and interstitial fluid function together in a unique way to protect against high levels of stress and strain, (Mow *et al*, 1989).

2.2 THE MECHANICAL PROPERTIES OF ARTICULAR CARTILAGE

Mow *et al* (1989) suggested that the biomechanical behaviour of articular cartilage could best be understood when the tissue is viewed as having two distinct phases: a fluid phase (the interstitial fluid) and a solid phase (the collagen-PG matrix). Articular cartilage can then be thought of as a fluid-filled, porous-permeable medium. Synovial joints can be subject to loads up to several times body weight (Weightman & Kempson, 1979) and under such physiological conditions articular cartilage must fulfill its two primary functions: to distribute the load, thus decreasing the contact stress, and to allow relative movement of the joint with minimal friction and wear.

2.2.1 The Viscoelastic Nature of Articular Cartilage

Articular cartilage is viscoelastic, i.e. its response to a constant load or deformation is time dependent. The compressive viscoelastic behaviour of articular cartilage is due primarily to the flow of the interstitial fluid (Weightman and Kempson, 1979, Kempson, 1979, Mow *et al*, 1989). Creep is caused by

exudation of the interstitial fluid which is initially rapid but diminishes gradually until fluid flow ceases and an equilibrium deformation is reached (figure 2.5). Initially, the flow of fluid is out of the cartilage owing to increased internal hydrostatic pressures generated by the compressive load. However, as the deformation of the cartilage increases the permeability of the tissue will decrease so restricting fluid flow, and the osmotic swelling pressure of the PG gel will gradually increase as interstitial fluid is removed. These factors help to reduce the rate at which fluid is exuded until no further deformation of the cartilage takes place, (Weightman & Kempson, 1979).

The viscoelastic stress relaxation response of articular cartilage to a constant deformation is associated with fluid redistribution within the porous solid matrix (figure 2.6). A high stress is generated by compaction of the solid matrix near the surface owing to exudation of fluid from the cartilage. Whilst the constant deformation is maintained fluid redistribution throughout the cartilage relieves the high compaction region near the surface giving rise to the stress relaxation phenomenon.

Articular cartilage, with a modulus of elasticity in compression approximately one twentieth of cancellous bone (Weightman & Kempson, 1979) deforms much more than bone. The viscoelastic nature of the response allows cartilage to behave differently under different loading

conditions, i.e. whether of short or long duration, and this, together with stress relaxation, ensures that there is an increase in the contact area and an even distribution of the stress within the cartilage so reducing the maximum stress applied to the subchondral bone.

2.2.2 The Permeability of Articular Cartilage

Articular cartilage is highly porous, but has a low permeability. High frictional resistive forces are generated when fluid is caused to flow through the porous matrix. The permeability of the matrix decreases exponentially as a function of increasing compressive strain and increasing applied fluid pressure, (Mow *et al*, 1989). Both of these factors cause compaction of the solid matrix which reduces the porosity and hence increases frictional resistance to fluid flow. Under high loads through the mechanism of increased frictional drag against interstitial fluid flow, the tissue appears stiffer and it is more difficult to cause fluid exudation.

2.2.3 Behaviour of Articular Cartilage in Tension

In tension articular cartilage is strongly anisotropic (being stiffer and stronger in directions parallel to the split line pattern) and inhomogeneous (being stiffer and stronger in the superficial region). These

characteristics are attributed to the varying collagen and PG structural organisation within the tissue. For example, the collagen-rich superficial zone apppears to provide the cartilage with a tough, wear-resistant, protective skin. Articular cartilage also exhibits viscoelastic behaviour in tension, which is associated with internal friction generated by polymer motion and the flow of the interstitial fluid, (Mow *et al*, 1989).

An example of the equilibrium stress-strain curve for a specimen of articular cartilage tested under a constant low strain rate (to ascertain the intrinsic mechanical properties of the solid matrix) is shown in figure 2.7. The tangent modulus (defined as the tangent to the stress-strain curve) results in a widely varying range for the Young's modulus of articular cartilage, 3-100MPa (Mow *et al*, 1989). However, at physiological strain levels (of less than 15%) the linear Young's modulus ranges from 5 to 10MPa, (Mow *et al*, 1989). Alteration of the collagen structure by reorganisation of the collagen fibres or a reduction of the cross-link density (such as in mild fibrillation or osteoarthritis (see section 2.4) changes the tensile properties of the cartilage, (Mow *et al*, 1989).

2.2.4 Behaviour of Articular Cartilage in Pure Shear

If articular cartilage is tested in pure shear under infinitesimal strain conditions, no pressure gradients

or volumetric changes will be produced within the material, and hence, no interstitial fluid flow will occur. A steady dynamic pure shear test can, therefore, be used to assess the intrinsic viscoelastic properties of the collagen-PG solid matrix. The magnitude of the dynamic shear modulus for normal bovine articular cartilage has been found to range from 1 to 3MPa, (Mow *et al*, 1989).

2.3 LUBRICATION OF ARTICULAR CARTILAGE

A healthy synovial joint permits pain-free movement under a wide range of loads and speeds, with very low frictional forces and very little wear of the cartilage surfaces. As stated earlier, one of the primary functions of articular cartilage is to provide such conditions for the joint to work. Such behaviour indicates that sophisticated lubrication processes are operative to reduce the friction and protect against wear. The lubrication mechanisms of articular cartilage have been subject to much investigation.

2.3.1 Historical Background to the Lubrication of Synovial Joints

Dowson *et al* (1981) provide a comprehensive overview of the historical background to the research into the lubrication of natural joints. MacConaill (1932) and Jones (1936) first proposed hydrodynamic fluid film

lubrication as the main lubrication mechanism. Charnley (1959) then produced evidence that suggested that boundary lubrication was important. McCutchen (1959) proposed the concept of "weeping lubrication" where the sponge-like cartilage generated a self-pressurized hydrostatic bearing. Dintenfass (1963) and Tanner (1966) proposed elastohydrodynamic lubrication which was supported by Dowson (1967) who concluded that the main lubrication mechanism was some form of elastohydrodynamic action determined by both sliding and squeeze-film action, with boundary lubrication providing the surface protection in cases of severe loading and little movement. Fein (1967) confirmed the presence of squeeze-film lubrication and Maroudas (1967) demonstrated the presence of gels formed on opposing cartilage surfaces, hence confirming the possibility of a boundary lubricant. Walker *et al* (1968) suggested the hyaluronate-protein complex in synovial fluid as the likely constituent of the boundary lubricant and also proposed "boosted lubrication" as a possible lubrication mechanism, where pools of synovial fluid were trapped in the surface contours of the articular cartilage with an increased concentration of hyaluronic acid. Unsworth *et al* (1975) confirmed that both fluid film and boundary lubrication mechanisms were present with associated squeeze-film action. Dowson and Jin (1986) then proposed their revolutionary micro-elastohydrodynamic lubrication theory. Prior to this elastohydrodynamic lubrication analysis predicted fluid films of about 0.1-1μm, but

healthy articular cartilage had an unloaded surface roughness of about 2-5μm (Gardner *et al*, 1971). Dowson and Jin (1986) coupled the overall elastohydrodynamic analysis of synovial joints with a study of the local micro-elastohydrodynamic action associated with the surface asperities. They concluded that the locally generated pressures smoothed the initially rough cartilage surfaces thus yielding fluid film thicknesses several times greater than the effective roughness of the opposing cartilage surfaces.

2.3.2 Lubrication Mechanisms Operative Under Varying Load Conditions

Synovial joints are subjected to a wide variety of loading conditions. The major weight bearing joints of the hip and knee, for example, sustain light loads during high-speed motions such as the swing phase of walking or running, impact loads of several times body weight during activities such as jumping or during heel strike in walking, and moderate fixed loads during prolonged standing. The lubricating processes responsible for the low friction and wear observed in such joints has been attributed to micro-elastohydrodynamic lubrication and to an absorbed boundary lubricant on the articular surface. Various combinations of such mechanisms are thought to be responsible for the full range of synovial joint lubrication.

Torzilli (1976) and Mow *et al* (1989) proposed that during normal gait exuded interstitial fluid would act as the lubricant. During prolonged static conditions exudation of the interstitial fluid would occur resulting in a squeeze-film action until the impinging cartilage surfaces were arrested by the formation of the gelled synovial fluid. Any further movement would then require boundary lubrication to occur initially. Load removal would then produce fluid reimbibition. Torzilli (1976) also postulated that as the joint moved, the loaded cartilage at the leading edge would undergo compression, and interstitial fluid would be exuded across the surface providing the lubricating fluid. In the trailing edge the previously exuded fluid would then be reimbibed.

2.4 DAMAGE OF ARTICULAR CARTILAGE AND OSTEOARTHRITIS

It is difficult to dissociate any discussion of potential mechanisms of damage of articular cartilage *in vivo* from the disease of osteoarthritis (OA) which is characterised by the destruction of this tissue. Indeed, many *in vitro* studies aimed to create an experimental animal model of OA in order to study the progression of such damage, (Radin *et al*, 1990). Post-mortem osteoarthritic cartilage has also been studied in detail as an example of *in vivo* cartilage degeneration, (Sokoloff, 1969, Freeman & Meachim, 1979).

Degeneration of articular cartilage is usually characterised by fibrillation of the articular surface (vertical splitting of the cartilage which eventually extends through the full depth). Transverse sections through examples of normal, mild and severely fibrillated cartilage are shown in figure 2.8.

2.4.1 Fragmentation of the Collagen Network

The most important failure-initiating factor is now generally considered to be fragmentation of the collagen fibre network, (Sokoloff, 1969, Freeman, 1975, Weightman & Kempson, 1979, Freeman & Meachim, 1979, Meachim, 1980, Muir, 1990, Mow *et al*, 1989). Freeman & Meachim (1979) postulated that the collagen network that originally crossed the split (fibrillation) in the cartilage must have ruptured. They provided morphological evidence that suggested that the splits initially occurred in the direction in which the network was weakest, i.e. parallel to the predominant fibre direction. Kempson (1979) showed that the tensile fracture stress, the static tensile stiffness and the tensile fatigue strength of articular cartilage decreased significantly with age. The tensile properties of cartilage have been shown to be related to the collagen fibre network (see section 2.2.3). These age-related changes, therefore, indicated that some element in the network changed with age making it more susceptible to mechanical damage. Freeman & Meachim (1979) stated that it was unlikely

that the fundamental event would involve the individual collagen fibres (as collagen turnover occurs very slowly, if at all), but would probably concern their "linkage" together to form a fibre network. Muir (1990) suggested that cleavage of type IX collagen (which is predominantly observed at intersections of type II collagen fibrils and is thought to have a role in stabilising the fibrillar network) by proteases in the tissue could weaken the collagenous network by removing some of its essential binding material.

The most popular hypothesis concerning the role of mechanical influences in the pathogenesis of fibrillation is that fatigue of the collagen network occurs owing to cyclic loading of the joint combined with a reduction in the tensile fatigue strength of articular cartilage with age, (Freeman, 1975, Swanson, 1976, Weightman & Kempson, 1979, Kempson, 1979, Freeman & Meachim, 1979, Mow *et al*, 1989). Kempson (1979) concluded from *in vitro* experiments that cartilage exhibited a typical tensile fatigue behaviour. He also provided evidence from the literature that cyclic compressive loading produced destruction of cartilage in a joint simulator, cartilage degeneration of living animals, and surface defects similar to early OA changes in some post-mortem human cartilage.

2.4.2 Loss of Proteoglycans and Increase in Water Content

Other principle changes that occur in OA cartilage include a decrease in the tissue PG content and a decrease in PG aggregation, so that the cartilage contains a larger proportion of unaggregated PGs and aggregates that are present are smaller than normal, (Brandt, 1985). Once the collagen network has been fragmented and the cartilage as a whole has, therefore, lost its tensile strength, PGs may leak from the matrix resulting in a decrease in stiffness and an increase in permeability (see section 2.1.2), (Freeman, 1975, Weightman & Kempson, 1979, Meachim, 1980, Mow *et al*, 1989). This process would be accelerated by a decrease in PG aggregation. The fragmented collagen network would also allow the abnormal swelling of cartilage, owing to increased water content, observed during the early stages of OA, (Sokoloff, 1969, Freeman & Meachim, 1979, Meachim, 1980, Freeman, 1980, Brandt, 1985, Muir, 1990).

2.4.3 Other Mechanisms of Cartilage Damage

Various other mechanisms of damage have been suggested. Several authors have postulated that repetitive impact loading is an important factor in the initiation of deep subsurface cracks, (Mow *et al*, 1989, Radin *et al*, 1990). Radin and Paul (1971) and Radin *et al* (1982) showed that repetitive impulsive loading and thickening of the

subchondral plate led to cartilage deterioration. Radin *et al* (1990) hypothesized from a survey of experimental animal models that loss of cartilage required shear stresses to be generated within the cartilage layer, not from rubbing, but from loading. They investigated this further with finite element models and postulated that shear strains can be generated internally by the deformation of the cartilage. Under a compressive load they suggested that the cartilage would spread laterally but is restrained at the bottom by the calcified bed. Shear stresses are thus generated deep in the cartilage and at the edges of the compressed zone which can result in deep horizontal splits in the cartilage. This in turn removes some of the constraints on the cartilage deformation thus potentially causing further damage to the collagen network. Rapid loading, as in repetitive impact loading, also prevents stress relaxation occurring in the cartilage (i.e. when water is allowed to flow away from the high pressure areas (see section 2.2.1)) thus reducing the cartilage deformation and minimising the contact area, so further increasing the stresses within the cartilage matrix, (Mow *et al*, 1989, Radin *et al*, 1990). Subchondral stiffening and advancement of the calcified zone, observed in degenerative joint diseases such as OA, could also have the effect of increasing shear strains deep within the cartilage. Radin *et al* (1990) suggested that resulting horizontal splits deep within the cartilage may combine

with vertical splits, allowing chunks of cartilage to break away and resulting in progressive cartilage loss.

Some workers (Sokoloff, 1969, Radin *et al*, 1980, and Bullough, 1990) argued that only a certain range of loading is healthy for articular cartilage and that above and below this range the cartilage suffers. Sokoloff (1969) suggested, with respect to low-loading damage, that insufficient mechanical stimulus causes disuse atrophy of the articular cartilage. This was based on observations of the thin appearance of tissue in paralysed extremities or in "unused" portions of diseased joints. He argued that lack of use would result in little movement of the interstitial fluid, and hence little circulation of metabolites to and from the chondrocytes. Fibrillation caused by such lack of use tends not to be progressive, i.e. the cartilage does not wear away. For cartilage fibrillation to become progressive it must be in a weight-bearing area, (Radin *et al*, 1980). Seedhom *et al* (1981) showed that the area where OA lesions occurred in the knee corresponded to contact areas which occurred when peak loads were transmitted. They showed that the stresses arose on two distinct levels: low stresses (1MPa) for approximately 90% of the time and high stresses (4-6MPa) for approximately 10% of the time. They proposed that this mode of stressing caused the cartilage to adapt chemically and mechanically to transmit the low stresses to which it is subjected for most of the time. When it

was subjected to the higher stresses, even for much shorter periods, it could not transmit them without sustaining some damage.

Mow *et al* (1989) argued that interfacial wear (adhesion or abrasion) would be unlikely in normal, healthy articular cartilage owing to the multiple modes of effective lubrication, but that these mechanisms may take place in an impaired or degenerated synovial joint. However, Swanson (1979) stated that there was no experimental evidence which showed with certainty that a failure of lubrication was or was not a causative factor in the first stages of cartilage degeneration. Brandt (1985) also supported this argument. Freeman and Meachim (1979) observed track marks on the cartilage surface parallel to the direction of movement of the joint and suggested that an abrasive wear mechanism could have been the cause.

2.4.4 Biochemical Factors

Metabolic abnormality was also a factor put forward to explain the reduction in PG content in the early stages of OA, (Freeman & Meachim, 1979, Kuettner *et al*, 1990). Kuettner (1990) argued that the integrity of normal articular cartilage is maintained by a fine balance between synthesis and breakdown of matrix components. In OA this is disturbed as the attempts to repair and maintain the matrix are outpaced by degenerative

changes. It was concluded that the chondrocytes appeared to play a major part in increasing the production of proteolytic enzymes which attack the surrounding matrix (possibly causing the initial fragmentation of the collagen network). Muir (1990) stated that even though synthesis continues as the collagen network is broken down the production of new collagen fibres is random, thus the former structure and strength of the collagen network is not restored.

As illustrated from the discussion above, the exact process by which articular cartilage degenerates has not yet been established. In particular, the initiating factor, that is whether mechanical stresses injure the chondrocyte thus causing it to release enzymes that instigate breakdown of the collagen fibre network, or whether mechanical influences alone initially damage the collagen network, has not been elucidated, (Brandt, 1985). However, as Meachim (1980) stated, the mechanical and biochemical hypotheses need not be mutually exclusive.

2.5 CRYSTAL DEPOSITION DISEASES

Dieppe and Calvert (1983) defined a crystal deposition disease as "a pathological condition associated with the presence of crystals which then contribute to the tissue damage". Synovial joints are particularly susceptible to crystal deposition which can then cause clinical

problems. Possible factors which may favour crystal deposition include: impaired removal of particles due to the acellularity of cartilage and poor transport properties, abnormal mechanical factors, synovial membrane inflammation and the loss of normal inhibitory mechanisms, (Dieppe & Calvert, 1983). Normal joint cartilage has a number of special factors which tend to inhibit the formation of bone mineral (hydroxyapatite) and other crystal deposits including: avascularity, macromolecular inhibitors and inorganic pyrophosphate. However, the mere presence of crystals is not bound to cause a clinical problem and many cases of crystal deposition in joints can be clinically asymptomatic, (Utsinger *et al*, 1975, Dieppe & Doherty, 1982, Dieppe & Calvert, 1983, and McCarty, 1986).

There are three known categories of particle that can produce damage in synovial joints. These are: (i) fragments of bone and cartilage, (ii) crystalline derivatives of normal body metabolites, e.g. monosodium urate monohydrate (MSU), calcium pyrophosphate dihydrate (CPPD) and calcium hydroxyapatite (HA) crystals and (iii) foreign particles introduced from outside the body, e.g. plant thorns and injected steroids, (Dieppe & Doherty, 1982, and Dieppe & Calvert, 1983). The important properties of these particles which determine their ability to cause damage include their hardness, solubility, size, shape and the physical and chemical properties of their surface, (Dieppe & Doherty, 1982).

Each of the three principal crystalline deposits (MSU, CPPD, and HA) can be associated with an acute self-limiting inflammatory reaction and with a chronic destructive form of joint disease, (Dieppe & Doherty, 1982, and Dieppe & Calvert, 1983).

2.5.1 Acute Inflammatory Diseases

Each crystal type (MSU, CPPD and HA) is associated with an acute inflammatory response; gout, pseudogout and acute calcific periarthritis, respectively. The crystals are found in the inflammatory exudate removed from the joint. However, all crystals have also been found in joint fluids in the absence of obvious clinical or pathological evidence of significant inflammation, (Dieppe & Doherty, 1982).

There has been much debate with respect to how the crystals come to be present in joint space (McCarty, 1972, Bennet *et al*, 1976, McCarty, 1977, Howell, 1980, Dieppe & Doherty, 1982, Dieppe & Calvert, 1983, Caswell *et al*, 1983, Schumacher *et al*, 1983, Howell, 1985, and McCarty, 1986). Two possibilities exist: (i) the crystals may arise from acute crystallisation within the synovial fluid, or (ii) crystals may be liberated from preformed deposits within the joint tissues, i.e. "crystal-shedding". Clinical and experimental evidence tends to support the "crystal-shedding" hypothesis. Dieppe and Doherty (1982) proposed three ways in which

CPPD crystals could be loosened from their cartilaginous matrix: (i) partial solubilisation of the crystals, (ii) biochemical alteration of the cartilaginous matrix, possibly by "enzymatic strip-mining" (McCarty, 1986), and (iii) mechanical disruption of the cartilage. However, which of the two proposed mechanisms actually operates to produce the acute attacks still remains to be determined. Dieppe and Doherty (1982) concluded that particles have a central role in some inflammatory joint diseases.

2.5.2 Chronic Destructive Joint Disease

All three crystal types (MSU, CPPD, and HA) have also been associated with destructive arthritis, typically osteoarthritis, although crystal deposits have been observed in cartilage with little or no apparent reaction, (Utsinger *et al*, 1975, Dieppe & Doherty, 1982, Dieppe & Calvert, 1983, and McCarty, 1986). Again, there has been much debate with respect to the relative association between crystal deposition and the disease of osteoarthritis, (Gerster *et al*, 1975, Huskisson *et al*, 1979, Howell, 1980, Dieppe & Doherty, 1982, Dieppe & Calvert, 1983, Doherty, 1983, Dieppe & Watt, 1985). Dieppe and Doherty (1982) quoted evidence that suggested that synovial fluid crystals, either HA, CPPD, or both, could be found in at least one-third of all OA cases. They listed four possible mechanisms of joint damage due to the presence of crystals: (i) synovial inflammation

due to the presence of particles causes the release of the destructive enzyme collagenase, (ii) altered cartilage resistance to mechanical forces owing to embedded mineral deposits, (iii) surface wear owing to the presence of particles in the synovial fluid, and (iv) altered cellular metabolism caused by the biologically active nature of the crystal surface. However, as stated previously, many people with radiological chondro-calcinosis (calcium crystal deposits in cartilage) have no other clinical signs of joint disease. Dieppe and Doherty (1982) proposed that crystal deposits behave as a potential "amplification loop" in chronic arthritis, in that joint damage due to various pathological factors may dispose to crystal deposition which then could accelerate cartilage damage, (figure 2.9).

The potential mechanical damage that crystals could cause to articular cartilage has only been touched upon in the literature. It has been suggested that crystal deposits within the cartilage could alter the biomechanical and biomaterials properties of the tissue leading to cartilage damage (Howell, 1980, and Dieppe & Doherty, 1982). Dieppe and Calvert (1983) and Dieppe and Watt (1985) postulated that crystal deposits alter the compliance of the cartilage structure thus reducing the load-spreading ability and, thus allowing the transmission of excess forces locally to the bone below. They suggested that fractures could develop in the

cartilage at the boundary between the relatively hard, inflexible deposit and the surrounding matrix. Dieppe and Calvert (1983) also postulated that localised loose aggregates may produce significant weakening of the cartilage resulting in local fracture and possible release of crystals. The potential for surface abrasion by wear particles accompanying OA or by hard, sharp crystalline particles has also been suggested, (Dieppe & Doherty, 1982, Dieppe & Calvert, 1983, and Dieppe & Watt, 1985, Ali, 1985). Ali (1985) suggested that increased deposition of HA nodules in the deeper zones of articular cartilage, resulting in an uneven subchondral region, would increase the stress on the articular cartilage when pressure was applied and hence, over a long period, the cartilage would degrade. Ali (1985) also suggested that the presence of numerous cuboid crystals observed in the surface region of articular cartilage would also increase the stress on the cartilage network, thus leading to eventual degeneration.

However, such postulations have been based upon clinical evidence alone. There have been no fundamental scientific investigations completed to support, or refute, any of these suggestions.

2.5.3 The Crystals

Dieppe and Calvert (1983) provided the most comprehensive study of the crystals associated with joint disease and their characterisations of the respective crystals are quoted here.

Gout is associated with crystals of monosodium urate monohydrate (MSU) of the triclinic form. They are needle-shaped 2-20μm long and 0.5-2μm wide. In any one fluid they are reported as being regular in size and shape. The crystals found within tophi or articular cartilage are similar, apart from they have a smooth surface, whereas those found in synovial fluid may have attached granular material which is thought to be protein, (Dieppe & Calvert, 1983). Joints with chronic gout often contain large deposits of urate on the surface of the cartilage, in the synovium and in the cartilage itself, (Dieppe & Calvert, 1983).

Chondrocalcinosis and pseudogout (calcium pyrophosphate dihydrate deposition disease) are both associated with the deposition of calcium pyrophosphate dihydrate (CPPD) crystals in the monoclinic or triclinic form, although the latter is the more common. Under polarised light the crystals appear as small rods or prisms 1-10μm long and 0.2-5μm wide. When examined by electron microscopy they vary considerably in size and shape, with the smallest being less than 1μm in length. CPPD crystals are found in articular cartilage, synovial fluid, the synovium and

the fibro-cartilage pads such as the meniscus. Within the articular cartilage crystal deposits are most commonly observed in the mid-zone where large aggregates are often formed.

Hydroxyapatite (HA) crystals have been associated with acute calcific periarthritis and with osteoarthritis. The two main sites of deposition are the periarticular tendons, articular cartilage and synovial fluid. The material generally looks amorphous under polarised light microscopy where individual crystals are too small to be identified. When using electron microscopy several different forms can be seen: (i) small crystals 0.01-1μm in length, (ii) small nodules of needle-shaped HA crystals in articular cartilage and synovial fluid, 0.1-1μm in diameter, (iii) rather larger ovoid smooth-surfaced bodies varying in size from 0.1-5μm in diameter where definite crystals cannot be identified, and (iv) amorphous or crystalline particles varying in size and shape. Dieppe and Calvert (1983) observed these crystals to be located in the mid- to lower zones of articular cartilage. Small plate-like HA crystals were also observed on abnormal articular surfaces. Ali & Griffiths (1983) and Marante *et al* (1983) also reported the presence of cuboid HA (or possibly whitlockite) crystals in the surface zone of arthritic articular cartilage.

Several other crystal types have been observed in synovial fluid or articular cartilage including brushite (Dieppe & Calvert, 1983, and Gaucher *et al*, 1978),

cholesterol (Dieppe & Calvert, 1983, Zuckner *et al*, 1964) and calcium oxalate (Reginato, 1989). Mixtures of different crystals present in the same joint have also been reported, (Halverson & Ryan, 1988, and Halverson *et al*, 1990).

2.6 SUMMARY

Crystals are recognised clinically to be associated with a range of joint diseases. Many investigators have speculated that their presence contributes mechanically, as well as biochemically, towards much of the damage observed. Previous studies from the mechanical perspective have been limited (Sutro, 1962, Lipshitz & Glimcher, 1979, and Clift *et al*, 1989). It was, therefore, considered appropriate to conduct a comprehensive investigation of the damage caused to articular cartilage by crystals in a controlled *in vitro* situation.

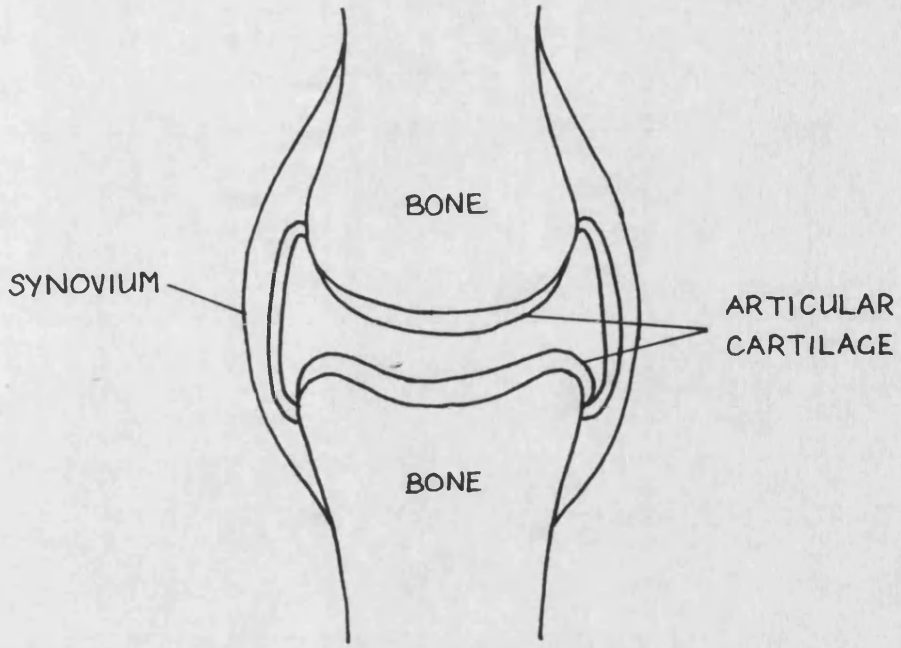


Fig. 2.1 A schematic diagram of a synovial joint.

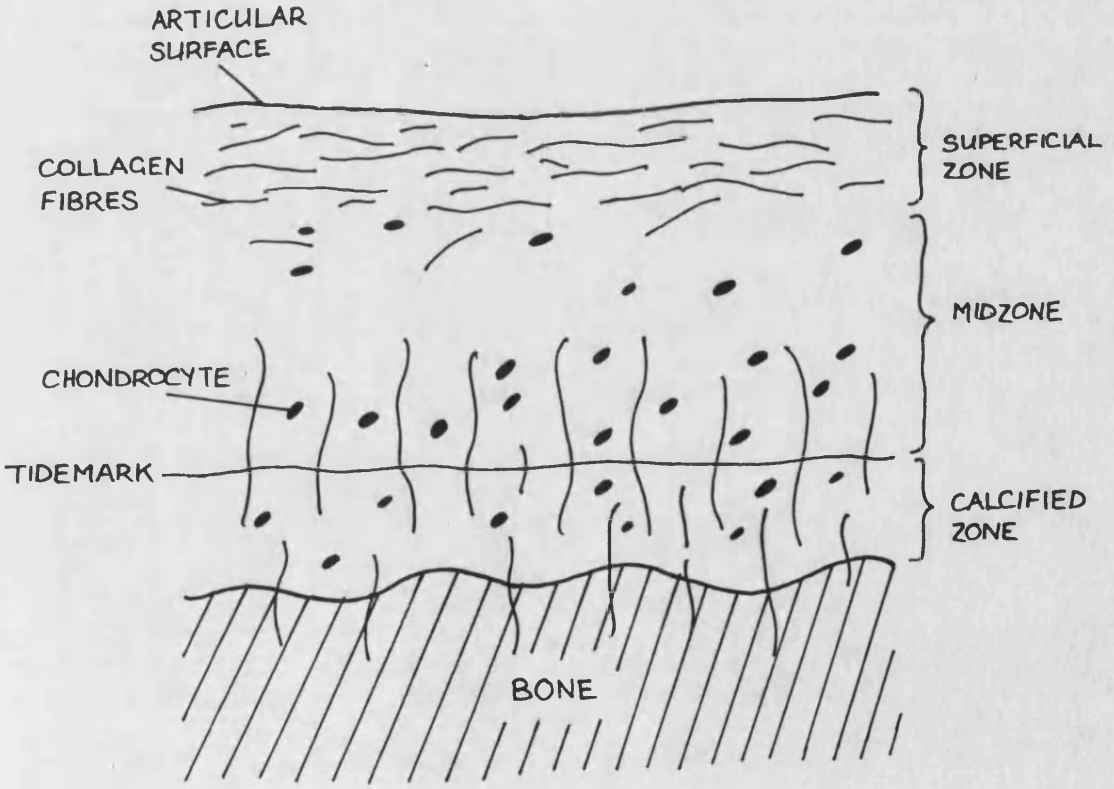


Fig. 2.2 A schematic diagram of the zonal arrangement of collagen fibres within articular cartilage. (After Dieppe & Calvert, 1983).

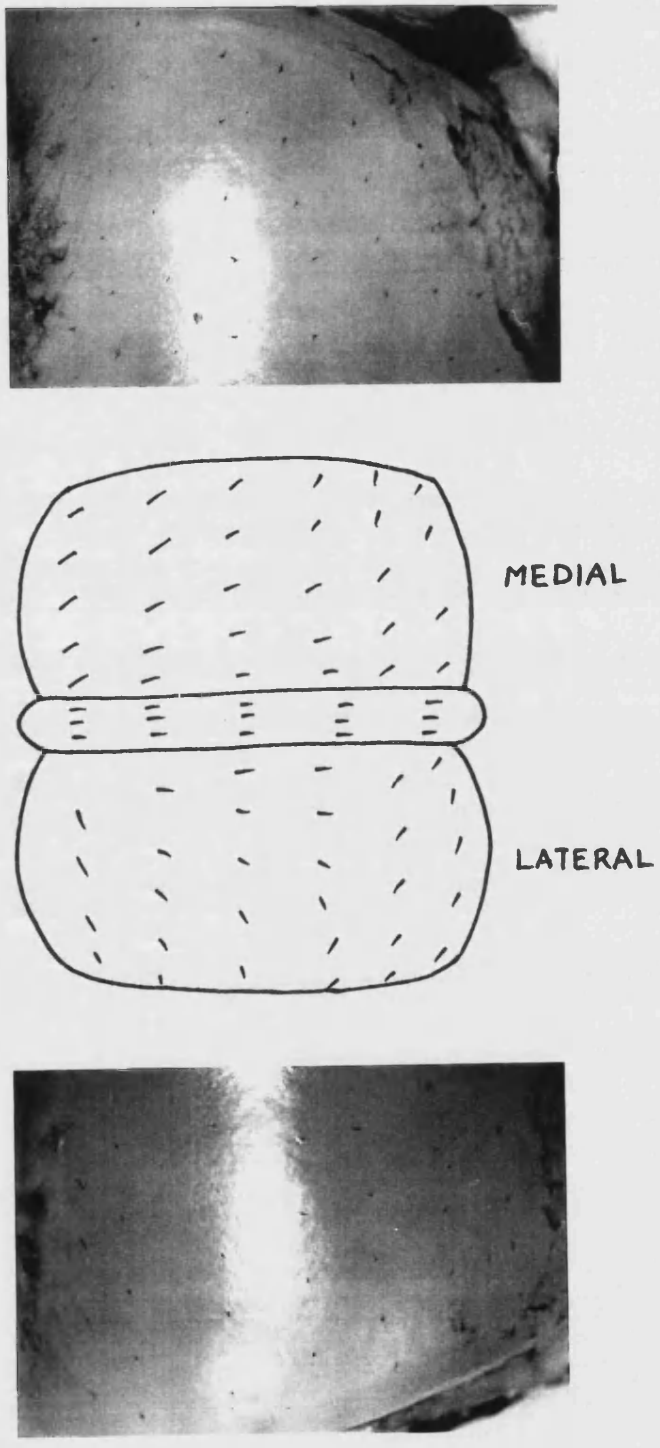


Fig. 2.3 The split line pattern for an equine fetlock joint.

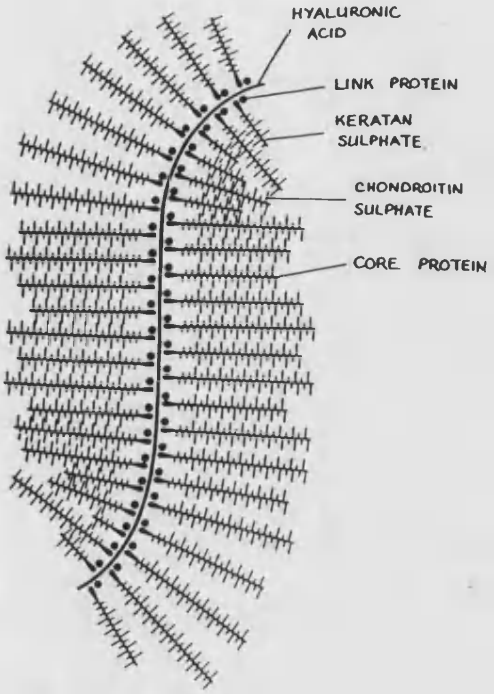


Fig. 2.4 A schematic diagram of a proteoglycan aggregate.

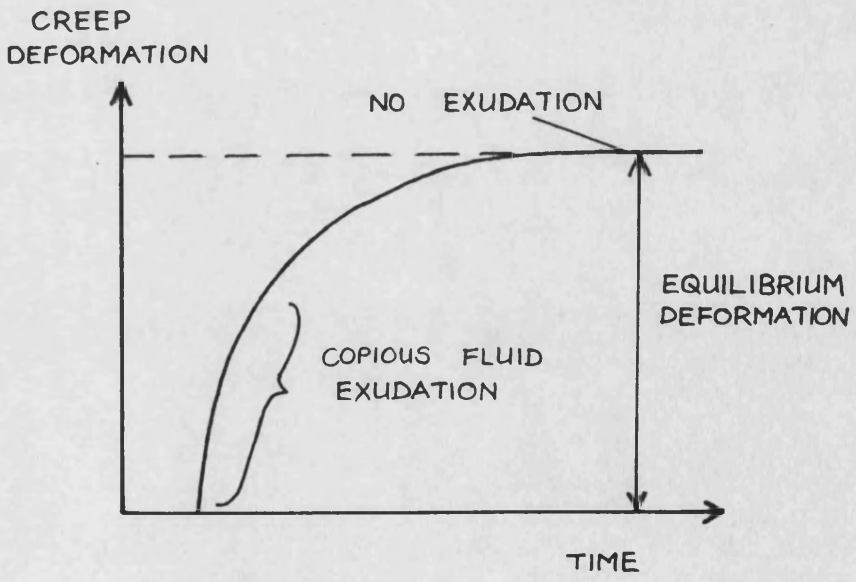


Fig. 2.5 A typical creep curve for articular cartilage. (After Nordin & Frankel, 1989.)

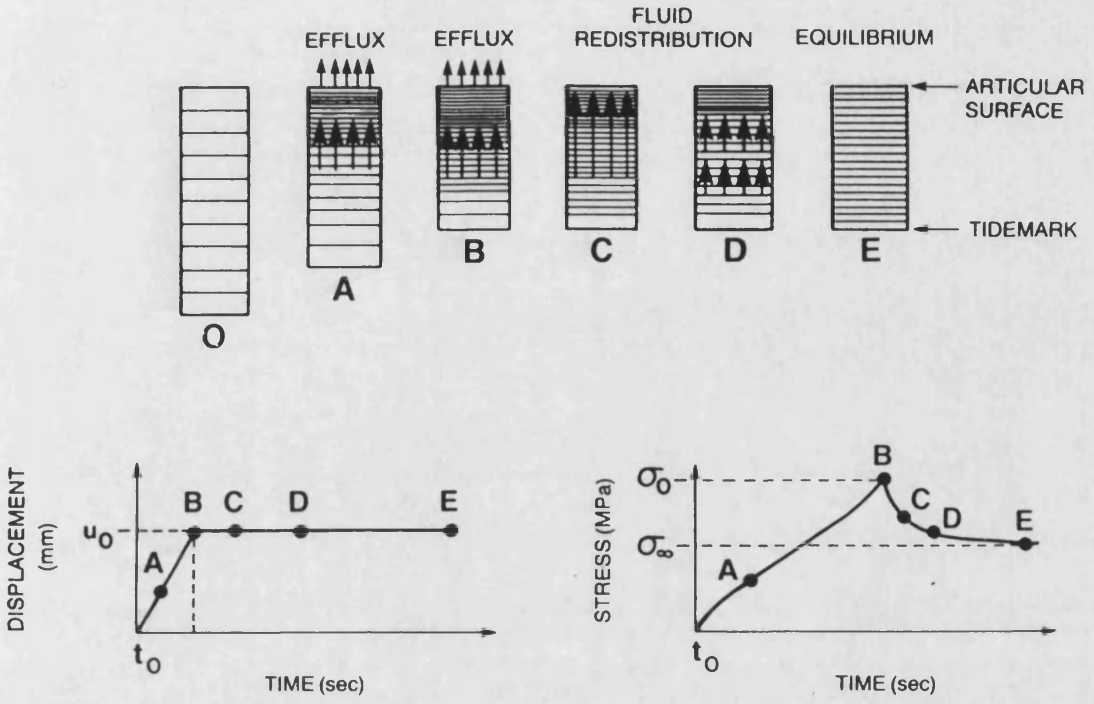


Fig.2.6 Schematic diagrams illustrating the viscoelastic stress relaxation response of articular cartilage to a constant deformation. (After Nordin & Frankel, 1989.)

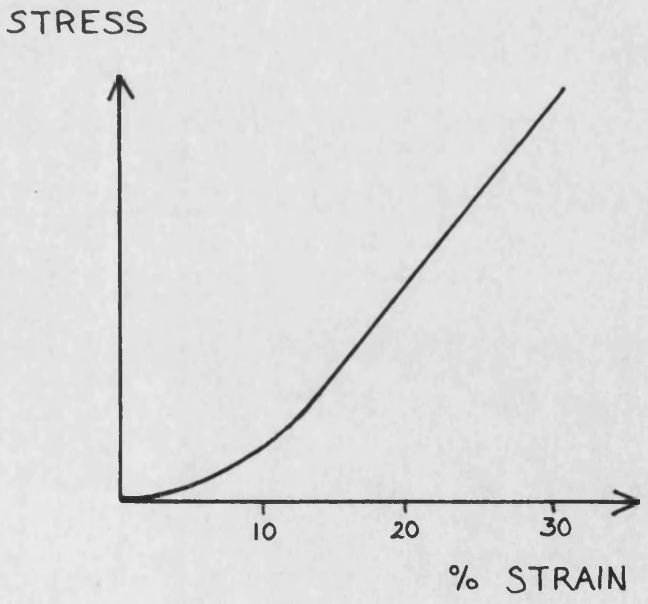


Fig. 2.7 A typical tensile stress-strain curve for articular cartilage. (After Nordin & Frankel, 1989.)

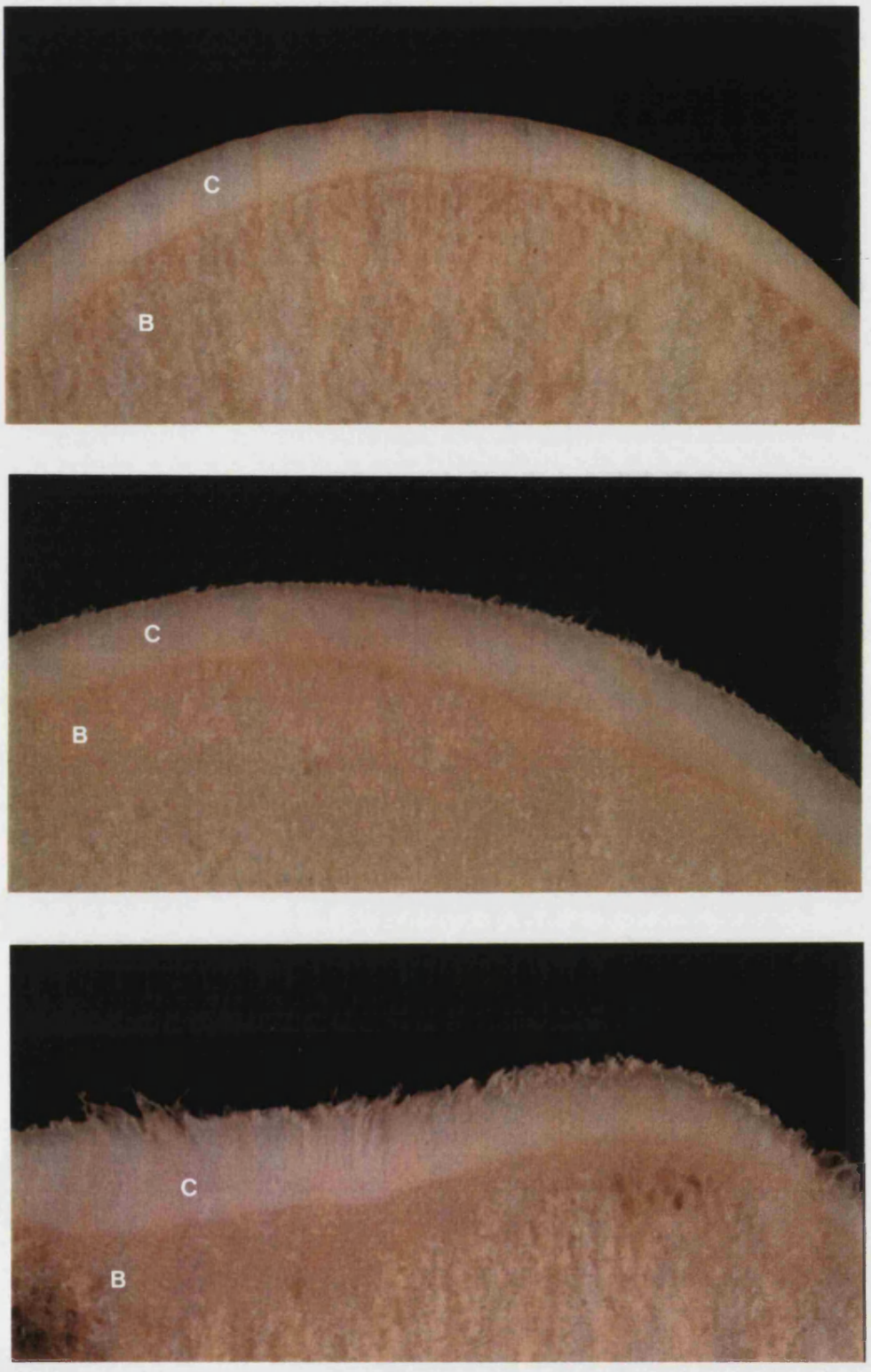


Fig. 2.8 Transverse sections through normal, mild and severely fibrillated cartilage. (C = cartilage and B = bone).

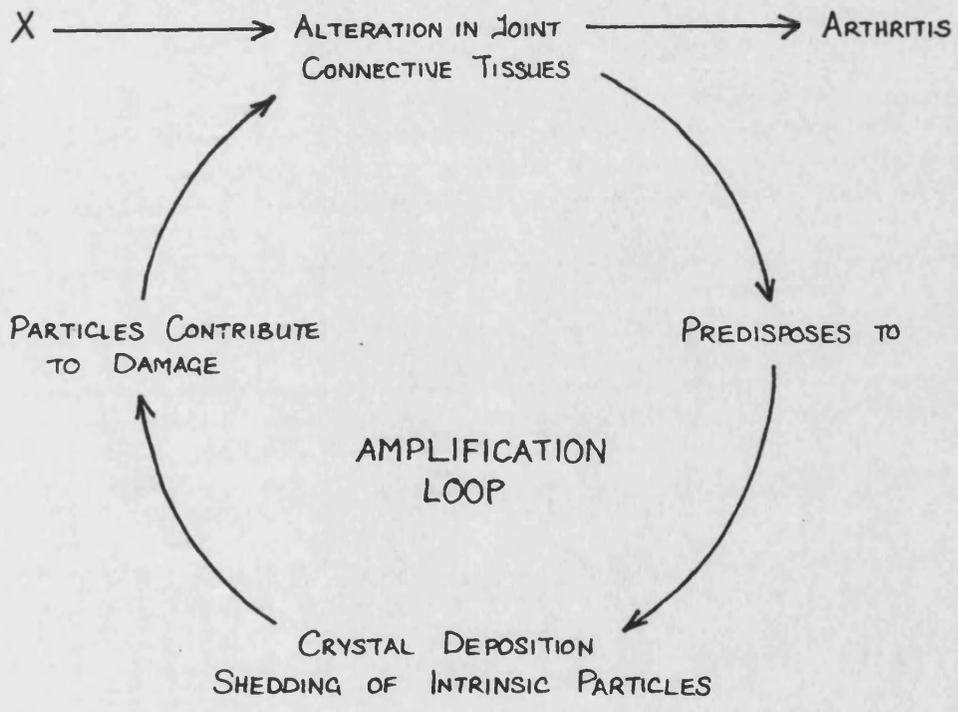


Fig. 2.9 The amplification pathway of particle-induced joint damage. (After Dieppe & Doherty, 1982.)

3. THE FRICTIONAL RESPONSE OF ARTICULAR CARTILAGE

3.1 INTRODUCTION

As previously discussed (section 2.5.5) there has been much debate with respect to the potential damage that crystals present on the articular surface may cause to the cartilage. This programme of research was initiated to investigate the frictional response of articular cartilage, in particular concentrating on the effect of crystals on the articular surface. It was assumed at this stage, that a high coefficient of friction would imply a high potential for damage. This work followed an initial study by Clift et al (1989) which compared the coefficient of friction of diseased post mortem cartilage with that of normal post mortem cartilage. Clift et al correlated the coefficient of friction of articular cartilage with the surface condition of the cartilage and the density of crystals within the surface zone. Further investigations, discussed here, attempted to quantify the frictional response of articular cartilage with and without artificially introduced surface crystals.

3.2 LITERATURE REVIEW

3.2.1 Values Previously Recorded for the Coefficient of Friction of Articular Cartilage

Typical values of the coefficient of friction of articular cartilage obtained by previous workers have been summarized by Swanson (1979). These are shown in tables 3.1 and 3.2. Table 3.3 gives some typical values for the coefficient of friction for various engineering systems (Swanson, 1979). It is clear from a comparison of these tables that even though the values measured for the coefficient of friction of articular cartilage are spread over two orders of magnitude they are significantly lower than many values commonly observed in engineering bearings.

Source	Joint	Coefficient of Friction
Jones (1934)	Horse stifle	0.02
Charnley (1959)	Human ankle	0.014, 0.024
Barnett *et al* (1962)	Dog ankle	0.018-0.03
Linn (1968)	Dog ankle	0.0044
Little *et al* (1969)	Human hip	0.003-0.015
Unsworth *et al* (1975)	Human hip	0.02-0.042

Table 3.1. Coefficients of friction measured in entire synovial joints. (After Swanson, 1979).

Source	Bearing materials	Coefficient of friction
McCutchen (1966)	Articular cartilage on bone, against glass	0.008-0.1
Dowson *et al* (1968)	Articular cartilage on bone, against glass, rubber or plastic	0.15-0.8
Walker *et al* (1970)	Articular cartilage on bone, against glass	0.0014-0.07

Table 3.2. Coefficients of friction measured on small pieces of cartilage. (After Swanson, 1979).

System	Coefficient of friction
Rubber tyre on dry road	1
Brake lining material against cast iron	0.4
Smooth metallic surfaces, specially cleaned and degassed	100
Steel shaft and bronze bush, oiled	0.2
Nylon against steel	0.3
PTFE against PTFE	0.07
Hydrodynamically lubricated bearing with oil	0.05
Hydrostatic air bearing	0.0005
Ball bearing	0.001

Table 3.3 Typical values of coefficients of friction. (After Swanson, 1979).

3.2.2 Experimental Details for Measuring the Coefficient of Friction of Articular Cartilage

In early reported studies whole synovial joints were used in order to obtain a measure of the coefficient of

friction (as shown in table 3.1). However, even though all of these investigators, apart from Jones (1936), took precautions to exclude the forces exerted by capsules and ligaments, these effects are unknown and as such the results are difficult to interpret. Swanson (1979), Barnett and Cobbold (1962) and Linn (1967) all state that the variability of results could partially be accounted for by the difficulty of calculating the coefficient of friction from measurements made on curved surfaces. Several workers attempted to alleviate this problem by using small specimens of articular cartilage, attached to subchondral bone, where relatively flat regions of cartilage could be tested against an opposing surface. However, in this case the specimen size was limited to a few millimetres diameter. Swanson argued that this too had its limitations as the flow of fluid through the short path between the centre of the loaded area and the edge of the specimen would be different from that in an intact joint. 'Edge effects' due to the unconstrained nature of the cartilage around the edge of the specimen could also have an unknown effect on the mechanical properties of the cartilage as a whole, and the frictional response in particular.

Another important factor to note from such a survey of the literature is that several different designs of apparatus were used, which must also contribute to the variability of the results reported in tables 3.1 and 3.2. Unsworth *et al* (1975) correlated the design of

apparatus with the mode of lubrication postulated by the authors. They noted from their survey of the literature that workers using pendulum machines to investigate the coefficient of friction of articular cartilage concluded that the mode of lubrication occurring at the fulcrum was boundary in nature, whereas those using reciprocating machines showed that fluid film lubrication was important. However, it is interesting to note that all of those workers who used pendulum machines also used entire synovial joints, whereas those testing small specimens of cartilage were necessarily using reciprocating machines.

Wide variations in experimental conditions, therefore, existed between different studies. These included: the size of specimen, the presence of subchondral bone, the load used, the relative sliding speed, the nature of the bearing surfaces, the curvature of the specimen, and the actual design of the apparatus. Data, summarised in table 3.4, clearly illustrate that there are many variables associated with measuring the coefficient of friction of articular cartilage which are likely to affect the final result. It may not always be meaningful, therefore, to compare directly results obtained from different methods, although comparative assessment within a study can be of use provided that the experimental conditions are carefully controlled.

Source (1st auth. only)	Load or Contact Stress	Speed	Lubri-cant	Coeff. of fric.	Bearing Material
Jones (1936)	13lb 5oz	0.8in/ min	Synovial fluid	0.114	Cart./ cart.
		0.2in/ min		0.022	
McCutchen (1962)	5lb	0.5mm/s	Water	0.1	Cart./ glass
			Synovial fluid	0.02	
Dowson (1967)	400lb/in^2 (2.8MPa)	1in/s	Synovial fluid	0.15	Cart./ glass
	50lb/in^2 (0.35MPa)	"		0.8	
Linn (1967)	40lb	40 cyc. /min	Synovial fluid	0.006	Cart./ cart.
		"	0.9% NaCl	0.012	
Little (1969)	200lb *(hip joint)*	-	Synovial fluid	0.008	Cart./ cart
			Ringers	0.013	
Malcolm (1973)	1-0.25MPa	1.25mm/s	0.9% NaCl	0.28	Plastic /cart.
Unsworth (1975)	133.5N *(hip joint)*	-	Synovial fluid	0.032	Cart./ cart.
	800N	-	"	0.015	
	1250N	-	"	0.008	
Zaki (1985)	5N	0.023m/s	Synovial fluid	0.26	Cart./ steel
	10N	"	"	0.18	Cart./ cart.
Ikeuchi (1990)	-	-	Saline	0.02	Cart./ cart.
				0.3	Cart./ glass

Table 3.4 A summary of data to illustrate the wide variation in loads (or contact stresses), relative speeds, type of lubricant and counterface material used to measure the coefficient of friction.

3.3 THE FRICTION APPARATUS

3.3.1 The Original Design of Friction Apparatus

The apparatus used in this study was basically the same as that used by Clift et al (1989) to measure the coefficient of friction of small specimens of articular cartilage. It was designed and constructed by Mr. D. Date of Rolls Royce and is shown in figure 3.1. The specimen was located in the specimen holder and secured by four screws. The holder was moved, with the aid of a thumb screw, under a hemispherical glass indentor (4mm diameter). The position of the counterweight determined the normal load on the glass hemisphere. The resistance to movement between the glass and the cartilage was then measured indirectly with a strain gauge arrangement, the signal being amplified and fed to a chart recorder. The system was calibrated with small weights of 10, 20 and 30g.

The resistance to movement was noted on the chart recorder tracing only when the glass indentor was normal to the cartilage surface. This position was judged by noting when the meniscus produced around the indentor was horizontal, and thus parallel, to the side of the carriage holder. A value for the coefficient of friction could therefore be calculated by dividing the value

measured for the resistance to movement by the normal load provided by the counter-weight;

$$\text{i.e. } \mu = F/N,$$

where μ is the dynamic coefficient of friction, F is the frictional force (resistance to movement), and N is the normal load.

3.3.2 Modifications to the Friction Apparatus

After a series of pilot investigations the original design of apparatus (described above) was found to be extremely limited and so several modifications were made. These were based upon a number of observations and experimental difficulties which will be discussed in more detail later. The modifications included: The replacement of the thumb screw with a small variable-speed motor; the replacement of the small hemispherical indentor with a cylindrical glass indentor of approximate radius 1", i.e. comparable to that of a femoral condyle; and the attachment of a pointer and ruler arrangement to the specimen holder to assist in the accurate location, and re-location for subsequent traverses, of the normal postion. All experiments described in this study included the use of the variable-speed motor and the pointer and ruler arrangement, although a variety of indentors were used.

3.4 MATERIALS

3.4.1 Source of Cartilage

Clift *et al* (1989) used post mortem articular cartilage from human knee joints, obtained from the Royal United Hospital, Bath, for their investigation of the frictional response of normal and diseased cartilage.

In further investigations, however, it was considered important to standardise the condition of the cartilage used in order to quantify the frictional response of crystals on the surface. A continuous supply of normal, undamaged cartilage was therefore required. Owing to the age of most human post mortem material it is not usually possible to obtain large quantities of normal human articular cartilage, and therefore an animal model was substituted. Initially, a bovine joint was dissected and tested. However, cows, like pigs and sheep, have two parallel bones in the lower part of the leg, hence the joints are quite small and not able to provide enough material to test. Horses, however, have a single bone in their lower leg and so even their lower joints provide substantial sized specimens. These joints were also readily available from a local abbatoir (Potters Horse Slaughterers, Bristol). The distal ends of the cannon and pastern bones were used. Each joint was cut to provide two specimens 2 x 3cm surface area attached to approximately 1.5cm of subchondral bone, as illustrated in figure 3.2.

3.4.2 Storage of the Specimens

All joints were initially stored frozen at -18°C within 2 hours of the sacrifice of the animal. These were later allowed to thaw overnight at room temperature. The distal ends of the cannon and pastern bones were then carefully dissected and specimens cut. After the specimen had been tested it was labelled and stored in formal saline solution.

Concern has been expressed in the past that freezing specimens of cartilage may alter their mechanical properties. However, previously published results (Hori (1973) and Kempson (1970)) indicate that the mechanical properties were not significantly affected by freezing.

3.4.3 Synthetic Crystals

Two types of synthetic crystal were used, calcium hydroxyapatite (HA) and calcium pyrophosphate dihydrate (CPPD). These two crystal types were chosen because they both exist in their natural form in arthritic joints suffering from crystal depostion diseases. All crystals were provided and characterised (unless otherwise stated) by infrared spectroscopy by Dr. P. Shellis of the MRC Dental Group, Bristol. Further chracterisation was completed at Bath with x-ray diffraction (XRD), scanning electron microscopy (SEM) and energy dispersive analysis (EDA). The XRD was carried out by Mr. B. Chapman of the

School of Physics, University of Bath, with a Philips (PW1010/80) x-ray generator with a copper target x-ray tube fitted with a nickel foil filter at a wavelength of 1.5418A. A Philips (PW 1024/10) Debye-Scherrer camera was used to obtain the film. Exposures were usually between four and six hours. The scanning electron microscopy was carried out on a JEOL-T330 or a JSM-35C microscope at an accelerating voltage of 10 or 15kV. Energy dispersive analysis, carried out in conjunction with SEM, was with a Link Systems AN10000 analyser attached to the JSM-35C microscope.

Calcium hydroxyapatite crystals (HA) were prepared following an adaptation of the method used by Okazaki *et al* (1984). Further analysis by XRD (see appendix, table A1) confirmed that the crystals were calcium hydroxyapatite. SEM (figure 3.3) and EDA (Ca:P = 1.43) were also completed. The crystals ranged in width from 0.5 to 2μm and in length from 1 to 5μm.

Calcium pyrophosphate dihydrate crystals (CPPD) were nominally supplied and characterised, again by infra-red spectroscopy, in their triclinic form by Mrs. Angela Swan of the Bristol Royal Infirmary. They were grown in an acrylamide gel following the technique of Harries *et al* (1983). These were also further analysed by XRD (see appendix, table A2) and SEM in conjunction with EDA. SEM revealed three different morphologies within the batch of CPPD crystals. These included rhomboid crystals, typical of triclinic CPPD (Mandel *et al*, 1988) (figure 3.4a),

wedged or arrow-headed crystals, typical of brushite (Gaucher *et al*, 1978) (figure 3.4b), and small rod-like crystals (figure 3.4c), which contained iodine as confirmed by EDA (see figure 3.5). X-ray diffraction (see appendix, table A2) proved inconclusive in positively identifying these crystals, but, it was thought that they had probably formed as a result of contamination from the washing of the 'CPPD' crystals in sodium periodate in order to dissolve the acrylamide gel in which they were grown. A second batch of crystals was therefore grown after which rigorous washing with CPPD-saturated distilled water eliminated the presence of the small rod-like crystals. The resulting triclinic CPPD crystals were used in friction tests completed by Hailey (1989).

3.5 EXPERIMENTAL METHOD

A similar general procedure to that of Clift et al (1989) was used to measure the coefficient of friction of small specimens of articular cartilage, but with several modifications. All tests were carried out under a normal load of 280g and a maximum speed of 0.7mm/s unless otherwise stated.

3.5.1 Location of the Normal Position

Clift et al (1989) located the normal position by eye, but, the accuracy of this method in the location, and re-location of the indentor after successive runs, was poor.

The following method for ascertaining the normal position was therefore followed. The specimen was securely attached in the holder. The indentor was then loaded upon the cartilage surface, and any horizontal force produced by the indentor slipping to one side or the other was recorded on the chart recorder via the strain gauge arrangement. The indentor was then lifted from the cartilage surface and the specimen, in the holder, was moved to the left or the right until no such horizontal movement was detected when the surface was reloaded. It was then assumed that the indentor was normally loaded upon the cartilage surface and this position was noted on a ruler by the position of the pointer. For subsequent traverses across the cartilage surface the chart recorder tracing was marked when the pointer reached the same position on the ruler. A mirror was used to eradicate any errors due to parallax. This method was found to be accurate to within 0.5mm when the 5V f.s.d. scale was used on the chart recorder.

3.5.2 Replacement of the Hemispherical Glass Indentor

After several experiments it became obvious that the use of the small hemispherical indentor was limited owing to uncharacterizable ploughing effects caused by its small size (4mm diameter). A subset of experiments was therefore completed in order to investigate a suitable replacement. First, the small indentor was replaced by half of a flat glass microscope slide attached to the

balance-arm. However, this arrangement did not enable the glass slide to be horizontal when placed on the cartilage surface and so the resistance to movement increased as the glass moved over the cartilage. It was, therefore, necessary to record the range of values for the coefficient of friction.

After consideration of the results obtained with the flat glass plate, it was decided that a large cylindrical indentor with a radius of curvature similar to that of a femoral condyle (approximately 1") would be used. This had the effect of providing a larger contact area, like the flat glass plate, and hence solved the problem of immersion of the small hemispherical indentor, but a curved surface was retained. At the time, this was considered to be more physiologically representative.

3.5.3 Quantification of the Frictional Response of Crystals on the Cartilage Surface

An experimental procedure similar to that used by Clift et al (1989) was followed, although with several important enhancements. Firstly, in order to standardise the amount of synovial fluid on the surface of the cartilage by removing any excess, each specimen was wiped slowly, for a period of five seconds with a clean tissue dipped in Ringer's solution. This was repeated three times, as described by Little et al (1969).

Each specimen was then checked for any signs of fibrillation by using an adaptation of the Indian ink staining technique desribed by Meachim (1972). Each specimen was removed from the Ringer's solution, and the cartilage surface was painted with neat Indian ink. The excess ink was then carefully washed away with a low pressure jet of distilled water. Any fibrillation on the surface of the articular cartilage remained stained with the Indian ink, and could clearly be seen with the naked eye. Any specimens that were abnormal were not included in the study.

Each specimen was oriented in the specimen holder so that the relative movement of the indentor on the cartilage surface would be aligned with the direction of movement occurring *in vivo*. Each specimen was then tested using the large cylindrical indentor to measure the coefficient of friction. Between six and twelve traverses of the cartilage were made so that a mean coefficient of friction could be calculated. The specimen remained in the specimen holder and was covered with a tissue moistened in Ringer's solution whilst a suitable deposition of crystals was produced for transfer onto the cartilage surface.

Crystal Deposition onto the Cartilage Surface

Many difficulties were encountered in retaining the crystals on the surface of the cartilage. After much consideration, the following procedure was used. Known

concentrations of hydroxyapatite suspensions in distilled water were made (0.05, 0.25, and 0.5mg/ml) and dispersed thoroughly in an ultrasonic bath for half an hour. 1ml of the suspension was then filtered slowly through a Swinnex-25 Millipore filter containing a 0.22μm membrane filter. This was followed by 1ml of 50/50 Alizarin Red S solution which stained the hydroxyapatite crystals a deep red. The filter was then flushed through with 2-3ml of distilled water, and 2ml of air to remove any excess fluid. Care was taken to achieve a slow constant rate of filtering in order to distribute the stained crystals evenly onto the membrane filter. The membrane filter with the crystals was then removed from the Swinnex-25 filter and placed, crystal-side up, onto a clean glass slide. The glass slide was then inverted over the cartilage specimen at approximately the normal position, and then pressed firmly onto the cartilage surface whilst being rotated.

This method proved reasonably succesful in transferring the red-stained crystal distribution from the membrane filter onto the cartilage surface. However, the crystals were not very securely located on the articular surface and great care had to be taken, when lubricating the cartilage, in order not to wash the crystals away. It was found adequate to place a drop of fluid gently onto the cartilage at the normal position: this did not then tend to flow away down the sides of the cartilage.

With a distribution of crystals now on the normal cartilage surface the specimen was retested, as for normal articular cartilage without crystals, in order to measure the mean coefficient of friction of the articular cartilage. This procedure was repeated for all three concentrations of crystal suspensions, and the coefficients of friction were compared for normal articular cartilage and articular cartilage with surface crystals.

3.5.4 Grading the Condition of the Bone-Cartilage Interface

Dieppe and Calvert (1983) and Sokoloff (1969) state that one classic feature of osteoarthritis is the protrusion of the bone into the cartilage at the interface. Radin et al (1973) also observed that the condition of the bone-cartilage interface was related to the condition of the articular cartilage in the local region.

The condition of the bone-cartilage interface for all human specimens (n=60) used by Clift et al (1989) was coarsely graded in this study as poor, medium or good by examining slab radiographs of each specimen under a light microscope (for enhanced resolution). The percentage of specimens in each bone-cartilage interface grade was then calculated according to the crystal density within the cartilage, and the fibrillation of the articular surface. These figures were then analysed in order to ascertain

whether any relationship between the condition of the bone-cartilage interface and the cartilage could be identified, as indicated in the literature.

3.6 RESULTS AND DISCUSSION

3.6.1 Ploughing Effects Caused By The Small Glass Indentor

During experiments with a small hemispherical glass indentor and a normal load of 280g (approximately 2.8N), it was noted that when testing soft or severely fibrillated specimens the indentor would often sink into the cartilage, sometimes almost disappearing. Consequently, a clearly visible track could be seen across the cartilage surface. This track took several minutes to disappear after the cartilage had been re-immersed in Ringer's solution. The possibility of using a lighter load for such specimens was therefore investigated. A preliminary experiment was completed to try and ascertain whether the coefficient of friction measured under these conditions was independent of the normal load.

The coefficient of friction was measured for two specimens 'a' and 'b' which were severely fibrillated and mildly fibrillated (but softer than expected), respectively, under a range of normal loads. The small

glass indentor was used for these experiments. The results obtained are given in table 3.5.

	Load /g	Mean Coefficient of Friction	Standard Deviation
a)	280	0.08	0.01
	240	0.12	0.05
	197.5	0.13	0.04
	155	0.12	0.03
	115	0.14	0.07
b)	280	0.34	0.01
	240	0.42	0.03
	197.5	0.48	0.05
	155	0.11	0.02
	115	0.11	0.03
	92.5	0.12	0.04

Table 3.5 (a) The mean coefficients of friction for specimen 'a' (severely fibrillated) measured for a range of normal loads.
(b) The mean coefficients of friction for specimen 'b' (mildly fibrillated, but soft) measured for a range of normal loads.

These data are far from conclusive. The results given in table 3.5(a), for severely fibrillated cartilage, suggest that the coefficient of friction is independent of the normal load (taking into consideration the standard deviations). However, in table 3.5(b), results for mildly fibrillated but soft cartilage clearly show that for loads of 155g, or less, the coefficient of friction was significantly lower than for loads of between 197.5 and 280g. More data are required before any firm conclusions

can be drawn but these results suggest that the coefficient of friction measured with the small indentor may be dependent on the degree, and type, of cartilage damage as well as the normal applied load. Practical experience of using this apparatus also indicated that the coefficient of friction was dependent on the amount by which the indentor was immersed in the cartilage.

Linn (1967) showed that the coefficient of friction of undamaged articular cartilage decreased as the normal load increased, in agreement with the established theory of the frictional properties of polymers (Steijn, 1967). However, if the deformation due to ploughing contributes a significant component of the measured friction, the measured value of μ would increase by a greater amount as the load increased. This trend is followed in the case of the mildly fibrillated but soft cartilage but not for the severely fibrillated cartilage. However, the assumption that cartilage deformation would increase with increasing load may not hold in the case of the severely fibrillated cartilage, as it can be imagined that the vertical clefts present in such a material would allow a small indentor to pass through with relatively low deformation compared to a soft but relatively intact surface (figure 3.6). Therefore, maybe it is not surprising that we do not see such a trend for the severely fibrillated cartilage.

It is also interesting to note that values measured for μ of the severely fibrillated cartilage are approximately the same as the lowest value measured for the mildly

fibrillated but soft cartilage. This also indicated that there was probably a greater degree of deformation occuring in the softer cartilage compared to the severely fibrillated cartilage and hence the higher values of μ. The coefficient of friction measured for the severely fibrillated cartilage in our study (0.08 for 280g) is also significantly lower than those values recorded by Clift et al (1989) (approximately 0.3) and as such appears to be untypical. However, it is postulated that the high coefficients of friction recorded by Clift et al (1989) may be affected by a high ploughing component in the sliding friction, produced by dragging the small indentor through the damaged cartilage.

The problem of the small hemispherical indentor disappearing in soft, fibrillated articular cartilage, and hence contributing an unknown ploughing component to the sliding friction, was therefore tackled from a different perspective by investigating suitable alternatives as the opposing surface to the cartilage in the friction apparatus.

3.6.2 Results Obtained Using a Flat Glass Plate

An obvious alternative to the small hemispherical glass indentor as the opposing surface to the cartilage was a flat glass plate. As described in section 3.5.2 the glass slide, attached to the balance-arm, was not horizontal and so a range of values for the coefficient of friction

had to be recorded. Two specimens were tested consecutively, first with the small glass indentor and then with the glass slide. A range of normal loads was used. Both samples were graded as mildly fibrillated with approximately 1/4 of the indentor immersed in the cartilage at a normal load of 280g.

The coefficients of friction for each specimen obtained by using both the indentor and the slide are given below in tables 3.6 and 3.7. The upper and lower values for the coefficients of friction from the glass plate have been quoted.

	Load /g	Mean Coefficient of Friction (μ)	Standard Deviation (σ)
Spec 1	280	0.18	0.02
	197.5	0.14	0.03
	115	0.16	0.05
Spec 2	280	0.27	0.05
	197.5	0.33	0.02
	115	0.41	0.04

Table 3.6 The mean coefficients of friction obtained with the small glass indentor for a range of normal loads.

	Load /g	Lower Limit μ	Lower Limit σ	Upper Limit μ	Upper Limit σ
Spec 1	280	0.04	0.02	0.07	0.01
	197.5	0.03	0.01	0.08	0
	115	0.03	0.01	0.08	0.02
Spec 2	280	0.06	0.01	0.09	0.01
	197.5	0.08	0.01	0.11	0
	115	0.04	0.01	0.09	0.01

Table 3.7. The mean coefficients of friction (μ) obtained with a flat glass slide for a range of normal loads, (σ is the standard deviation).

It is obvious from these results that the values of μ measured with the glass slide were significantly lower than those obtained with the indentor. The values from the glass slide were also within the range of values quoted by the majority of workers, as listed in section 3.2.1, whereas those measured with the indentor fell only within the range quoted by Dowson et al (1968). (Dowson et al measured μ for undamaged cartilage, however, Clift et al (1989) showed that with the apparatus described in this study there was no significant difference bewteen μ for normal and mildly fibrillated cartilage.) Both of these facts indicated that the higher values of μ obtained with the indentor could be due to an additional ploughing component caused by the indentor being immersed in the fibrillated cartilage, whereas the glass slide could be imagined as riding over the surface fibrillation. Furthermore, there appears to be no difference between the coefficients of friction for the

range of normal loads when using the flat glass slide. However, the results obtained from the glass indentor were still inconclusive with μ appearing to be independent of the normal load in the case of specimen 1, but increasing by approximatley 50% when the load was decreased from 280 to 115g in the case of specimen 2. This trend was in the opposite direction as shown in the previous experiment (described in section 3.6.1) for the mildly fibrillated specimen but is the same as that described by Linn (1967), in that μ decreased as the normal load increased. These results indicated that in addition to problems associated with the design of the apparatus there was significant variability in the quality of the cartilage specimens tested which was unable to be controlled.

It was interesting to note that the values measured for μ with the slide fell within the range of values quoted in the literature, when some sort of fluid film lubrication was assumed to be present (see section 3.2). Dowson et al (1968), however, stated that boundary lubrication conditions were present when they obtained values for μ of between 0.15 and 0.8. These compared with values obtained by Barnett and Cobbold (1962) and Clarke *et al* (1975) where coefficients of friction of 0.27 and 0.15, respectively, were measured with no fluid present. In the present study, even though the cartilage was reimbibed with liquid after each test, it could be argued that the friction experiments were completed dry as the cartilage

was not immersed in a fluid, in which case the values of μ measured by myself and Clift et al (1989) fell within the range defined by other workers for these conditions.

A siginificant disadvantage of using the glass slide was the low contact stresses associated with the low normal loads available and the relative increase in contact area compared to the indentor. The range of loads used with this apparatus (93.5 to 280g, equivalent to approximately 0.9 to 2.8N) had been recognised (Seedhom, 1988) as a limitation of its design. Physiological loads on the knee joint, for example, have a maximum of approximately three times body weight (body weight for an adult man is about 700N) during a normal walking cycle (Nordin & Frankel, 1989). The reduction in contact stresses associated with the slide was, therefore, considered to be a further limitation of the apparatus. It has been suggested that for any further work the normal load applied to the cartilage should be increased. However, the frictional effect of an increased load on the linear bearings on which the specimen holder rests was thought to be too great when considering physiologically representative loads.

3.6.3 Effect of Motor Speed on the Coefficient of Friction Measured With a Flat Glass Plate

Another limitation of the apparatus identified by Seedhom (1988) was the low speeds associated with the relative

movement of the cartilage and the indentor. The speed of the carriage holder could be controlled by varying the speed of the motor controlling the screw, (see section 3.3.2). An isolated experiment was completed where the relationship between the coefficient of friction and the motor speed was established when the flat glass plate was used. The results are tabulated below in table 3.8.

Spec	Motor Speed mm/s	Lower Limit μ	Lower Limit σ	Upper Limit μ	Upper Limit σ
1	0.14	0.04	0	0.08	0.01
2	0.37	0.04	0.01	0.07	0.01
3	0.56	0.04	0	0.07	0.01
4	0.73	0.04	0.01	0.06	0.01

Table 3.8. The relationship between the motor speed and the mean coefficient of friction (μ), (σ is the standard deviation).

These results clearly show that when the flat glass slide was used over the range of motor, or carriage, speeds available, the coefficient of friction was independent of the sliding speed. These data were contradictory to the evidence supplied by Linn (1967) that the coefficient of friction of articular cartilage decreased with increased speeds and that of standard polymer theory as described by Swanson and Freeman (1977). It was concluded, however, that the similarity of the values of μ measured in this study was probably due to the very limited range of speeds used (0.14 to 0.73mm/s). These speeds were also

extremely low compared to physiological values of about 80mm/s.

It was suggested that further work should consider an additional modification to the apparatus described in section 3.3, in order that a greater range of physiological speeds could be used to measure the coefficient of friction of articular cartilage. The practical problem of visually noting the position of the pointer at such speeds would be overcome if a flat glass plate were substituted for the spherical or cylindrical indentors.

3.6.4 Use of a Large Cylindrical Indentor

A further problem with the simple arrangement of the slide being attached directly to the balance-arm was that it was not horizontal (see figure 3.7), hence the range of results quoted in table 3.7. This, however, did not have an easy solution. The angle of the balance-arm, and hence the glass slide, was dependent on the height of the specimen in the holder. It was, therefore, no trivial matter to have a system that would allow variable adjustment of the angle of the slide in order to set it horizontally for each specimen. The alternative was to transpose the specimen and the slide so that the cartilage ran against the glass rather than vice versa, as the apparatus was set up. This too, though, proved no trivial matter. It was eventually decided that, rather

than sustain involved alterations of the apparatus, a large cylindrical indentor with a radius of curvature similar to that of a femoral condyle (approximatley 1") would be used. This, like the glass slide, had a larger contact area and thus solved the problem of immersion in damaged cartilage. A single value for μ was recorded, as for the small indentor, at the normal position.

3.6.5 Effect of Curvature of the Specimen and Starting Position on the Coefficient of Friction

Hailey (1989) completed experiments with the modified friction apparatus (i.e. with the large cylindrical indentor) that indicated that the degree of curvature of the specimen and hence the starting position of the indentor on the curved specimen directly influenced the value recorded for the coefficient of friction at the normal position.

Considering values obtained by other investigators for the coefficient of friction of cartilage from entire specimens, as listed in section 3.2.1, Swanson (1979) suggested that the differences between results could be partially explained by the difficulty of calculating μ from measurements made on curved surfaces. Evidence that the coefficient of friction depends on the curvature of the specimen has also been provided by Barnett and Cobbold (1962) and Linn (1967). Seedhom (1988) suggested that the differing curvatures of specimens used in our

apparatus would also influence the values obtained for the coefficient of friction. Indeed, Hailey (1989) showed that the coefficient of friction was dependent on the curvature of the specimen and also on the starting position of the cylindrical indentor on the curved specimen.

3.6.6 Quantitative Analysis of the Frictional Response of Crystals on the Surface of Articular Cartilage

Normal equine (horse) articular cartilage was used to quantify the frictional response of cartilage with crystals on the articular surface. The cartilage was initially tested without any additional crystals present and a value for the coefficient of friction obtained. A measured concentration of crystals was then filtered through a millipore filter, and the crystals deposited on the filter membrane were transferred to the cartilage surface, as described in section 3.4.3. A basic measure of the density of crystals on the surface of the articular cartilage was thus obtained.

Three concentrations of hydroxyapatite crystals were used, and the values for the mean coefficients of friction were calculated for each of these. The results obtained are tabulated in table 3.9, and presented graphically in figure 3.8. Each individual point represents the mean coefficient of friction of one specimen. The standard deviations are also shown. Each

vertical pair of points represents one specimen, first tested without added crystals present, and then tested with hydroxyapatite crystals on the articular surface.

Conc. of Crystals mg/ml	Specimens 1		2		3	
	μ	σ	μ	σ	μ	σ
Normal 0.05	0.103	0.006	0.075	0.008	0.076	0.016
	0.097	0	0.080	0.013	0.076	0.002
Normal 0.25	0.102	0.006	0.077	0.008		
	0.141	0.013	0.114	0.018		
Normal 0.5	0.042	0.003	0.085	0.010	0.072	0.006
	0.242	0.053	0.233	-	0.308	-

Table 3.9. Pairs of results comparing the coefficient of friction (μ) of normal equine articular cartilage with and without surface hydroxyapatite crystals. (σ is the standard deviation).

Figure 3.8 suggested a direct correlation between the coefficient of friction and the concentration of hydroxyapatite crystals on the surface of the articular cartilage. Apart from the 0.05mg/ml concentration the coefficient of friction with surface crystals present is significantly greater at the 0.2% level (using Student's t-test) than that for normal cartilage without surface crystals.

When measuring the coefficient of friction with the highest concentration of crystals on the articular

surface, 0.5mg/ml, it was noted that the value measured for the coefficient of friction gradually fell as the testing procedure continued. This is illustrated in figure 3.9. One possible explanation for this, could be that if some superficial crystals were washed away, the concentration of crystals on the articular surface, would fall throughout the testing procedure, and μ would approach that of normal articular cartilage without surface crystals. The results used in figure 3.8 for the concentration of 0.5mg/ml were based upon the highest value recorded.

These results suggested that the coefficient of friction increased with the density of HA crystals on the surface of the articular cartilage although accurate determination of the quantity of crystals on the surface was not achieved. Interestingly, Hailey (1989) repeated these experiments with synthetic CPPD crystals and found a *decrease* in the coefficient of friction with an increase in crystal concentration on the articular surface, compared to that for articular cartilage with no crystals. It was suggested that the CPPD crystals, with a lower aspect ratio than the HA crystals could have acted like roller bearings between the two opposing surfaces thus having the effect of reducing the coefficient of friction, (Schweitz and Ahman, 1986) whereas the needle-like HA crystals were more likely to stick into the articular surface thus increasing the coefficient of friction (see figure 3.8).

Hailey (1989) also observed that in the case of the CPPD crystals the coefficient of friction *increased* during the test towards the value obtained for normal cartilage without crystals, compared to the situation for the HA crystals where the coefficient of friction decreased during the test procedure towards the normal value. This observation by Hailey (1989) confirmed the suggestion that the superficial crystals were gradually being washed from the surface of the cartilage, so that the measured μ value returned to that obtained for normal cartilage without crystals present.

3.6.7 Relationship Between the Bone-Cartilage Interface, the Crystal Density within the Cartilage, and the Fibrillation of the Cartilage

The radiographs of the specimens tested by Clift et al (1989) were re-examined, and the condition of the bone-cartilage interface was graded as poor, medium or good. The percentage of specimens in each bone-cartilage interface grade, calculated according to the crystal density within the cartilage, and the degree of fibrillation of the articular surface are tabulated in tables 3.10 and 3.11, and graphically illustrated in figures 3.10 and 3.11.

	Good	Medium	Poor
No Crystals	8%	4%	9%
Crystal Grade 1	56%	58%	55%
Crystal Grade 2	32%	38%	0%
Crystal Grade 3	4%	0%	36%
Sample Size	25	24	11

Table 3.10. Percentage of specimens in each bone-cartilage interface grade according to the crystal density within the cartilage.

	Good	Medium	Poor
Normal	52%	22%	0%
Mild	35%	65%	50%
Severe	13%	13 %	50%

Table 3.11. Percentage of specimens in each bone-cartilage interface grade according to the degree of fibrillation of the articular cartilage.

These results suggested that the worse the condition of the bone-cartilage interface, the higher the crystal concentration in the cartilage, and the greater the degree of fibrillation. They, therefore, appeared to confirm the hypothesis that there was a correlation between the local condition of the bone-cartilage interface and that of the cartilage as suggested by Radin et al (1973).

3.7 CONCLUSIONS AND FURTHER DEVELOPMENT

Data obtained in the investigation of the frictional response of crystals present on the articular surface were limited owing to problems encountered with the design of the apparatus. However, results indicated that the coefficient of friction increased with an increasing concentration of HA crystals present on the articular surface but decreased with an increasing concentration of CPPD crystals (Hailey, 1989). It was suggested that this could be related to their different morphologies and aspect ratios, the CPPD crystals acting like "roller bearings" between the two opposing surfaces of cartilage and glass, and thus lowering the coefficient of friction compared to that of articular cartilage without crystals and that with HA crystals. The implications of these results, though, are limited due to the constraints of the apparatus; tests being carried out at low loads (280g) and low speeds (0.7mm/s). Also, the effect of the curvature of the specimen and the importance of standardising the starting postion between tests had not been appreciated at this stage. This could account for some of the variability in the results shown in figure 3.8.

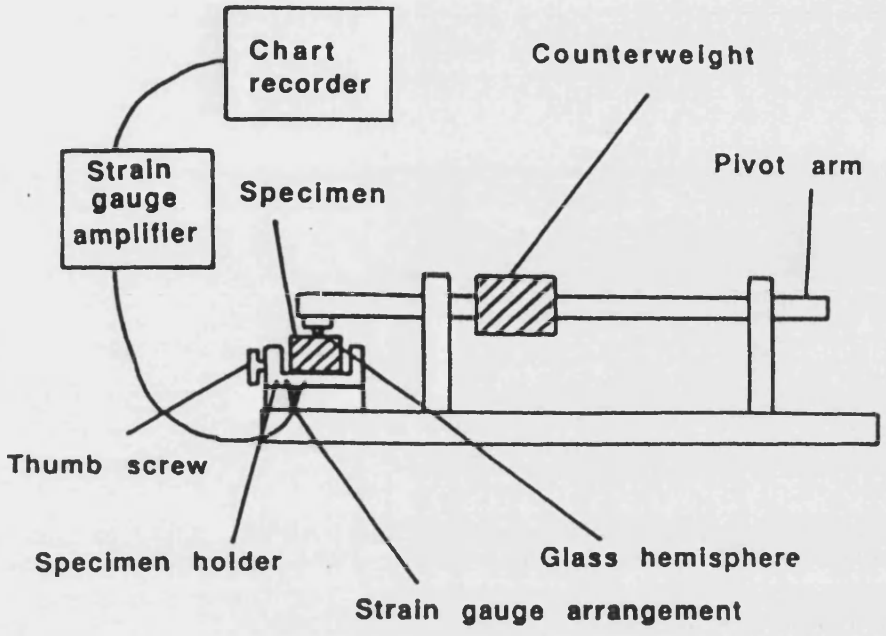
It was concluded after extensive investigation that the apparatus, described in section 3.3, was not suitable in that form for further study of the frictional response of articular cartilage and the effect of crystals on the

articular surface. Several potential modifications were investigated including an increase in the loads used, an increase in the range and value of speeds used and the use of a horizontal flat glass slide instead of a small hemispherical glass indentor or, as used later, a large cylindrical glass indentor. However, the apparatus described in section 3.3 was not capable of supporting such drastic modifications to its design.

The aims, objectives and methodology of the project were therefore reconsidered. It was accepted that this particular piece of research had provided valuable experience of, firstly, handling and testing unfixed biological tissue in a mechanical testing environment, but also of experimenting with an unstandardised, variable material such as articular cartilage. The original aim of the project, however, was to investigate the damaging potential of crystals on the articular surface. It was argued at this stage that an investigation of the wear caused by such crystals to the cartilage rather than the frictional response would provide a more fundamental approach to this problem.

It had been assumed when measuring the frictional response of crystals on the surface of articular cartilage that a direct correlation existed between the coefficient of friction and potential damage to the cartilage, i.e. that a high coefficient of friction indicated a greater potential for damage. However, such a relationship has never been established in the case of

friction (Swanson, 1979, and Freeman & Meachim, 1979), although one does exist in the case of wear, i.e. a high measure of wear indicates a high degree of damage to the material. It was therefore concluded that further research would investigate the effect of crystals in the lubricant on the wear of articular cartilage.



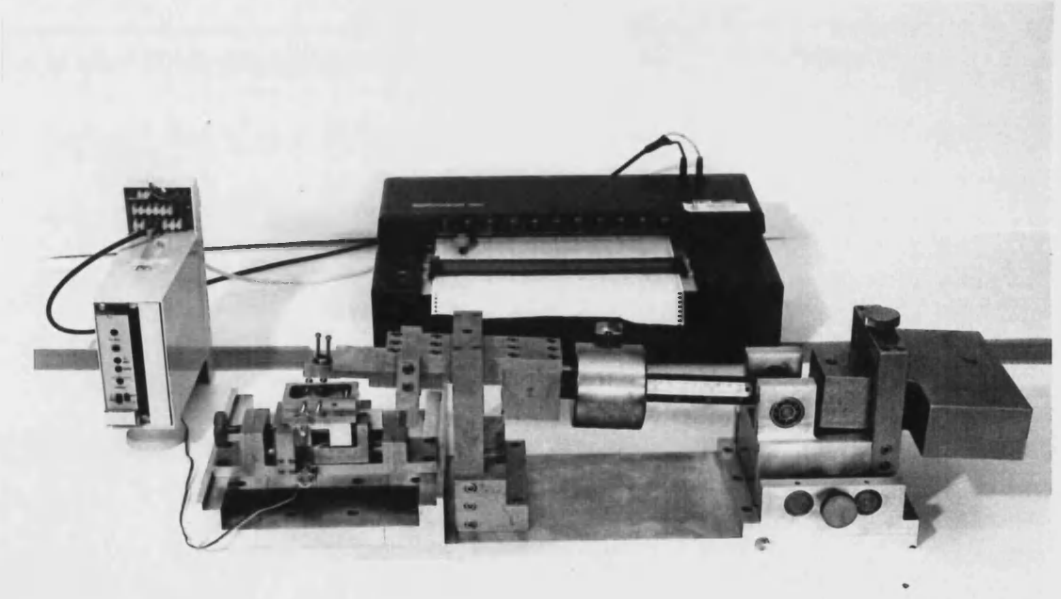


Fig. 3.1 The friction apparatus.

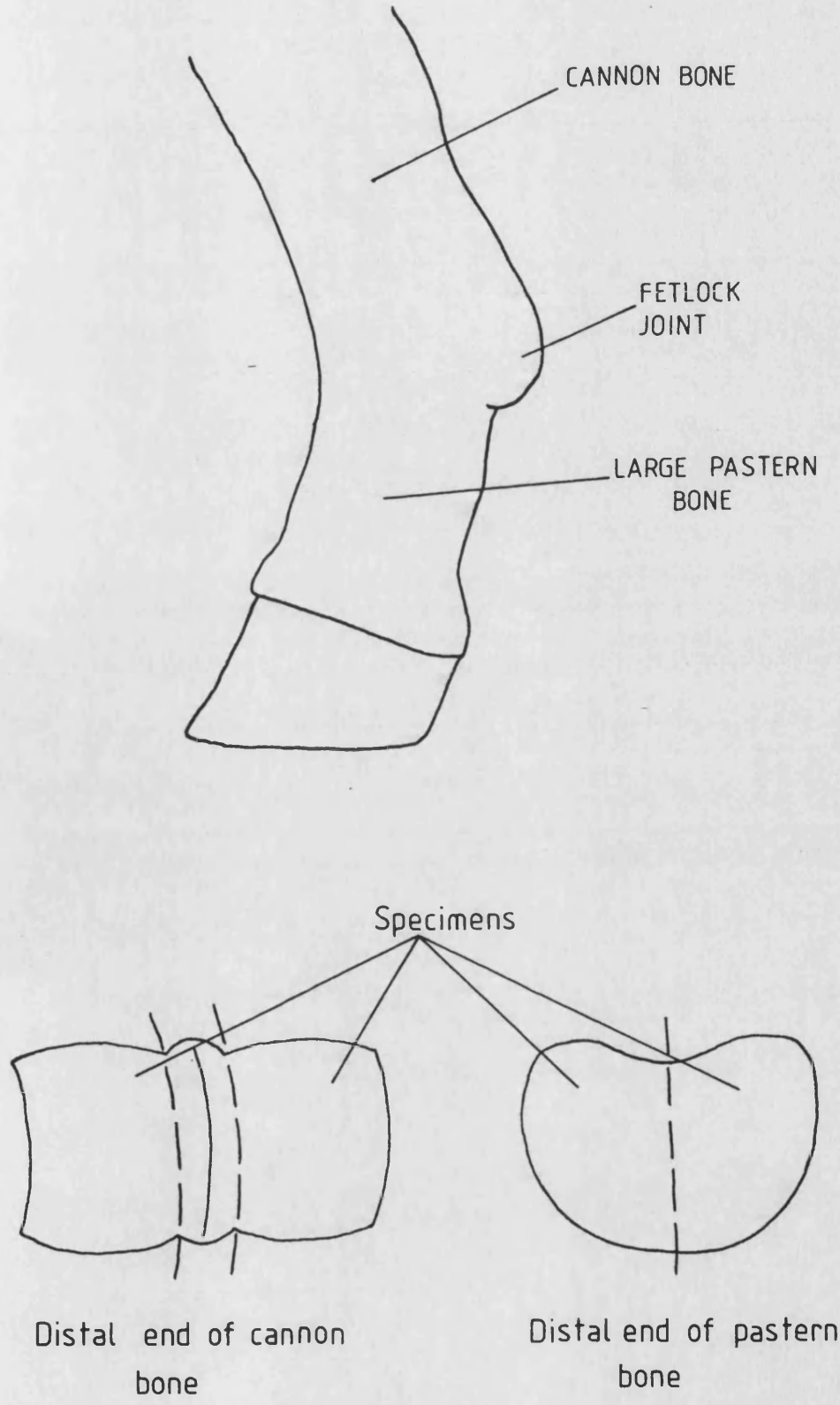


Fig. 3.2 A schematic diagram illustrating the specimens cut from equine pastern and fetlock joints.

Fig. 3.3 SEM of the HA crystals used in the friction experiments.

Fig. 3.4 SEMs of the "CPPD" crystals used in the friction experiments: a) typical t-CPPD crystals, b) typical brushite crystals, and c) small rod-like crystals containing iodine.

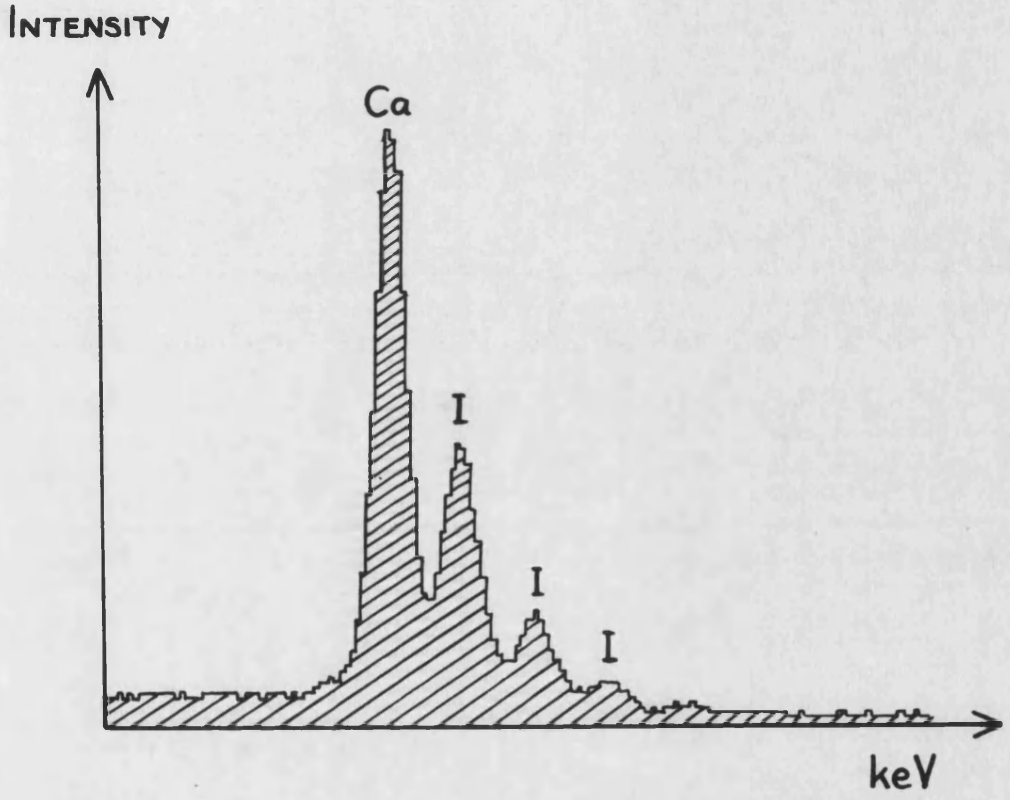


Fig. 3.5 EDA of the small rod-like "CPPD" crystals identifying iodine as part of their composition.

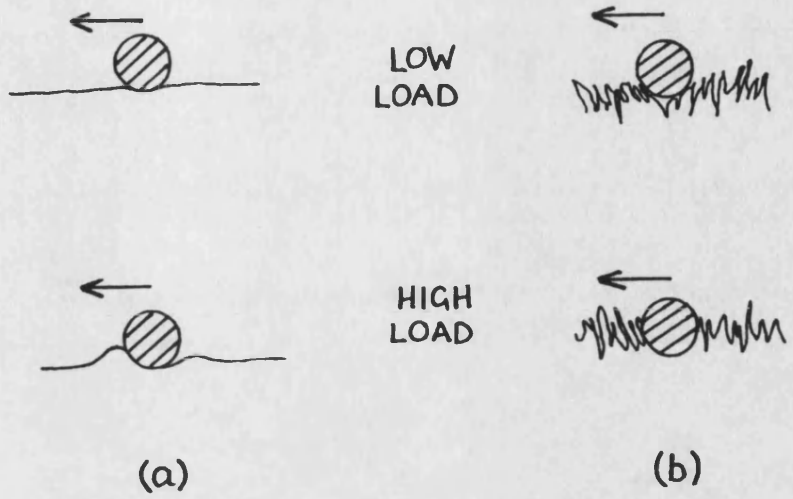


Fig.3.6 Schematic diagrams illustrating the relative deformation of cartilage for a) a soft but relatively intact surface, and b) a severely fibrillated surface.

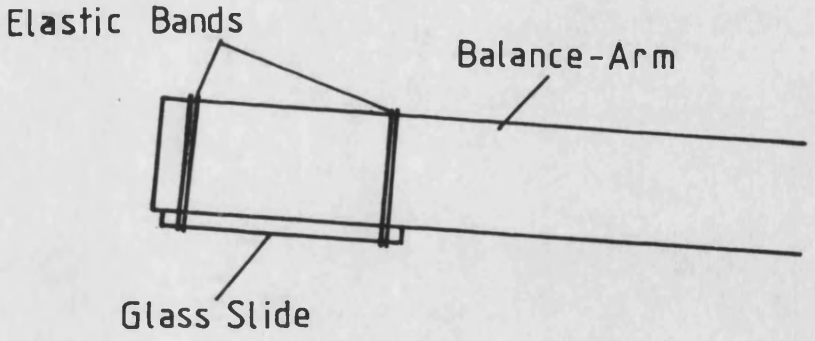


Fig. 3.7 A schematic diagram of the attachment of the flat glass slide to the balance arm.

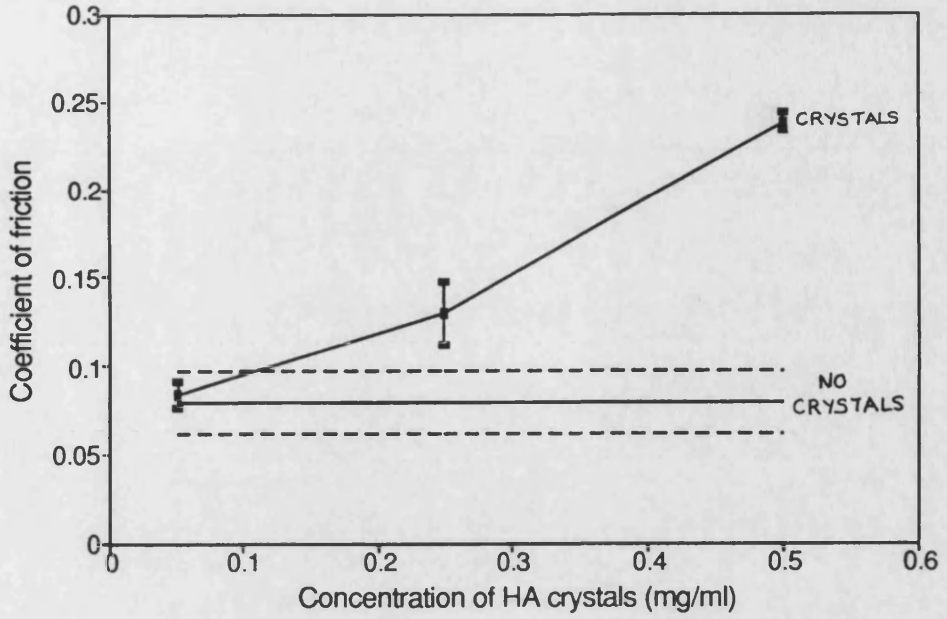


Fig. 3.8 The mean coefficient of friction and standard error for three concentrations of HA crystals on the articular surface compared to the average mean and standard error for cartilage with no surface crystals.

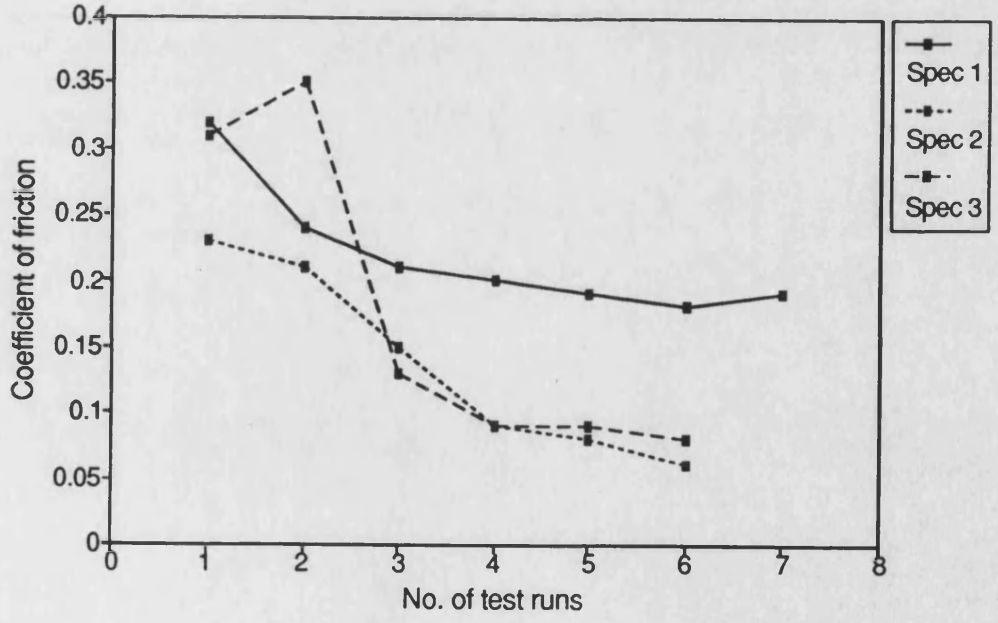


Fig.3.9 The coefficient of friction for consecutive test runs for a concentration of 0.5mg/ml HA crystals.

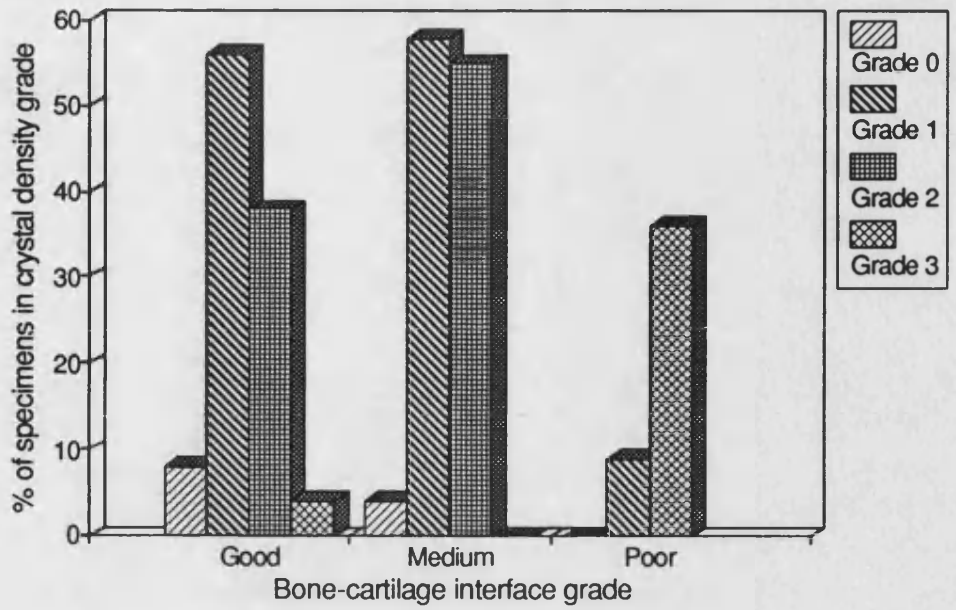


Fig. 3.10 The percentage of specimens in each bone-cartilage interface grade according to the crystal density within the cartilage.

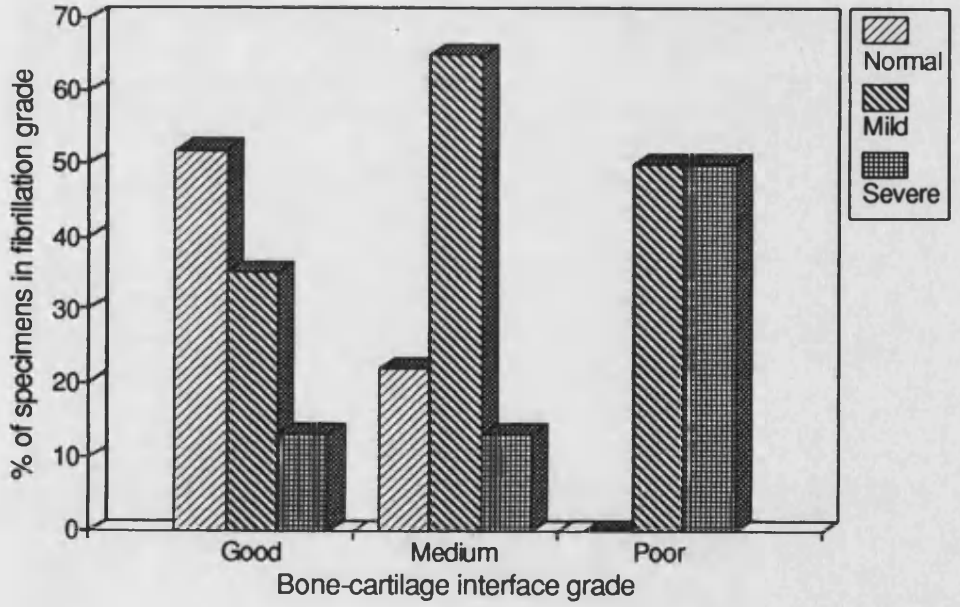


Fig. 3.11 The percentage of specimens in each bone-cartilage interface grade according to the degree of fibrillation of the articular surface.

4. THE WEAR RESPONSE OF ARTICULAR CARTILAGE

4.1 INTRODUCTION

Arthritic diseases that affect the joint, such as osteoarthritis, are generally characterised by the eventual destruction of the articular cartilage (see section 2.4). The cause of this degeneration is, as yet, unclear but it is thought to be due to a combination of biochemical and mechanical factors (Sokoloff, 1969, Freeman & Meachim, 1979, Brandt, 1985). Much research has been completed in the study of the biochemical factors (Sokoloff, 1969), but little work (see section 2.4 and 4.2), in comparison, has been reported where potential mechanical influences have been investigated.

Some arthritic diseases are associated with the deposition of crystals in the synovial fluid or articular cartilage of the joint. Perhaps the best known example is that of gout, where long needle-like crystals are characteristically found in the synovial fluid of the big toe. Various other crystals are also observed (see section 2.5.3), the most common being calcium pyrophosphate dihydrate and calcium hydroxyapatite. Dieppe and Calvert (1983) defined a crystal deposition disease as a pathological condition associated with the presence of crystals which then contribute to the tissue damage. Such damage often includes inflammation of the

surrounding synovial membrane, but mechanical factors also play an important role in any resulting acute and chronic joint damage, including surface abrasion of the articular cartilage (Freeman & Meachim, 1979, Dieppe & Calvert, 1983). This chapter will attempt to address some of the factors that are important in the mechanism of damage caused by surface abrasion of the cartilage by crystals. Such an approach has been only briefly reported in the literature by two other research groups (Sutro, 1962 and Lipshitz & Glimcher, 1979). The aim of the investigation reported here was to test the hypothesis that crystals present in synovial fluid could cause abrasive damage of the articular surface by investigating the effect of crystals in a lubricant on the wear of articular cartilage.

Wear is defined as the progressive loss of substance from the operating surface of a body and it occurs as a result of relative motion at the surface. Numerous factors affect the wear of any given material including the surface chemistry, the surface roughness, the load, the frictional forces, the contact area, the lubricant, the mechanical properties of the material, the presence of any hard inclusions, and the mechanism by which wear is achieved. The study of the wear properties of materials has, therefore, generally been empirical. Equations describing the laws of wear always contain factors which have to be determined by experiment.

Arnell et al (1991) argued that these are inherently imprecise.

Wear mechanisms operative in normal and pathological joints have not been totally elucidated owing to "the experimental difficulties, if not impossibility, of isolating the variables that affect the tissue's wear, in vivo, and the difficulties of measuring wear rates under reproducible conditions" (Lipshitz & Etheredge, 1984). The wear of articular cartilage in any environment other than that of a synovial joint, however, will necessarily be dependent on factors that do not exist in vivo. To overcome some of these problems and to allow greater control of some of the important factors a simple experimental system designed to control as many variables as possible was used in this study. (This is discussed in more detail in section 4.3.) The argument presented by Lipshitz and Glimcher (1979) that "it was felt that an understanding of the wear characteristics of articular cartilage under these conditions, where each of a number of variables can be independently varied, will be helpful in understanding the effect of various factors on the wear behaviour of the tissue under in vivo conditions" was subscribed to in this program of research. It was anticipated, that results obtained with such a system would further our general understanding of the wear response of articular cartilage with crystals present in the lubricant.

In addition to testing normal, undamaged cartilage in the wear apparatus, cartilage that had been scratched to varying degrees in vivo was also tested. This provided a direct comparison between the wear behaviour of normal and damaged cartilage. It was anticipated that results obtained from such experiments would provide an indication of the relative susceptibility of previously damaged cartilage to further wear processes.

4.2 LITERATURE REVIEW

The difficulties of studying the wear of articular cartilage under conditions that are physiologically representative have already been mentioned (section 4.1). Some investigators, though, did attempt to simulate such conditions by testing whole joints either in live animals (Radin et al, 1973, and Seirig & Gerath, 1975) or taken post-mortem (Barnett, 1956, Radin & Paul, 1971, Clarke et al, 1975 and Radin et al, 1982). All such experiments were necessarily conducted in pendulum-type apparatus, and both contacting surfaces were of cartilage. The lubricant varied, with most investigators either using the original joint synovial fluid or replacing this with a standardised buffer or saline solution. One group (Clarke et al, 1975) ran the joints dry in an attempt to accelerate the wear process. Several researchers (Barnett, 1956 and Radin & Paul, 1971) also investigated the effect of different loading regimes on the cartilage, i.e. oscillatory, compressive

and impulsive. The physiological conditions of load and speed vary between joints and between animals and for many are not well documented. The experimental conditions set by all of these investigators were not, therefore, necessarily physiological and since many variables were not controlled the interpretation of these data was not trivial.

Other researchers, notably the group comprising, variously, Lipshitz, Etheredge and Glimcher, but also Simon (1971), used isolated specimens of articular cartilage attached to subchondral bone and concentrated on controlling and independently varying important factors, such as the geometric contact areas and contact stresses, without a direct attempt to duplicate in vivo conditions. One of the main criticisms of such work is that the cartilage was worn against surfaces very different from that in the joint, (a stainless steel plate in the case of Lipshitz et al, and a fine metal rotary file in the case of Simon , 1971). The frictional forces and stress distribution would, therefore, also be quite different from those existing in vivo.

Both approaches, i.e. with either whole joints or cartilage specimens, were subject to the natural resistance of cartilage to wear. As Clarke et al (1975) stated so succinctly: "The joint mechanisms which may have to perform efficiently for a period of twenty to one hundred years have proved to be wear resistant during tests which ranged from periods of several hours

to days"! Various mechanisms were used to try and accelerate the naturally slow wear rate of the cartilage including running the joint dry (Clarke et al, 1975), the use of a load regime just lower than that which was discovered to damage the subchondral bone (Radin & Paul, 1971), the use of a counterface other than normal articular cartilage (Lipshitz, Etheredge and Glimcher), and the use of a small rotary file run backwards (Simon, 1971). A summary of these reports is given in table 4.1.

The measurement of the wear of articular cartilage also proved to be a problem. Owing to the variable fluid content of cartilage, techniques for measuring wear that are used with more conventional materials, such as recording the weight loss of the specimen or any dimensional changes, are not sufficiently accurate. Owing to the small amounts of cartilage usually removed, as well as the variable fluid content and hydrophilic nature of the material, collection and weighing of the wear debris would also be inaccurate. The most successful and sensitive method seemed to be that employed by Lipshitz et al, where a biochemical analysis of the hydroxyproline content (derived from the collagen in the cartilage) in the lubricant was used as a quantitative measure of the wear of cartilage. Simon (1971) and Radin et al (1982) used a measure of the depth of cartilage worn or the deformation of the cartilage as a measure of wear. Simon also attempted to collect, dry and weigh the debris. All other investigators used a combination of visual, optical and

scanning electron microscopy to evaluate the condition of the articulating surface qualitatively.

Source	Specimen	Counter -face	Lubri -cant	Load	Speed
Barnett (1956)	rabbit ankles	cart.	SF - HA	1.4 & 2.5kg	100 cyc/min
Radin & Paul (1971)	bovine meta-carpal-phal.	cart.	SF & buffer	1000lb static & 500lb static + 500lb impulse	40 cyc/ min
Simon (1971)	human patella & canine humeral heads	fine rotary file	saline & SF	284 & 71 psi falling to 5-10psi	120rev /min
Clarke et al (1975)	human hip joints	cart.	none	623N, 801N	40 cyc /min
Seirig & Gerath (1975)	patella joint of live rats	cart.	SF	0.45, 0.9, 1.8kg	1500 cyc/ min
Lipshitz, Etheredge & Glimcher (1976)	proteo-glycan extract. cart.	stain-less steel	phos-phate buffer	0.69-6.9MPa	52cm/ min
Lipshitz, Stone & Glimcher (1978)	flat cart. plugs	formal-dehyde react. cart.	phos-phate buffer	1.16-3.51MPa	52cm/ min

Source	Specimen	Counter -face	Lubri -cant	Load	Speed
Lipshitz & Glimcher (1979)	flat cart. plugs	stain-less steel	saline soln.	1.65MPa	0-50cm/min
Lipshitz et al (1980)	flat cart. plugs	stain-less steel	Tris-HCl	1.7MPa	52cm/min
Radin et al (1982)	bovine meta-tarso-phal.	cart.	SF & buffer	125kg static + 125kg impulse	40 cyc/min
Lipshitz & Etheredge (1984)	flat cart. plugs	stain-less steel & formal-dehyde reacted cart.	Tris	0.69-6.9MPa	52cm/min

Table 4.1 Summary of the literature investigating the wear of articular cartilage in vitro. (SF = synovial fluid, HA = hyaluronic acid.)

A number of observations were made in these studies with respect to the degree of damage caused under various experimental conditions. However, only Lipshitz et al attempted to provide a detailed explanation of the phenomena observed. Lipshitz and Glimcher (1979) wore flat cartilage plugs (attached to subchondral bone) against stainless steel plates with varying degrees of surface roughness. They assumed that the wear mechanism between the cartilage and the relatively smooth plates

(one with a mirror finish) was generally adhesive in nature. The wear rates in this instance gradually decreased with time until a constant linear wear rate was achieved which was dependent on the contact stress. They suggested that reorientation of the macromolecular domains in the same direction as that of the wear could explain the gradual reduction of the wear rate with time. However, when cartilage was worn against rougher plates a linear wear rate was produced straight away. It was assumed that the wear mechanism in this case would be abrasive in nature and reorientation of the macromolecules would not occur to the same extent. A later study by Lipshitz, Etheredge and Glimcher (1980) on the wear characteristics of chemically modified articular cartilage concluded that the wear of cartilage was also dependent on the extent of the interactions between the constituents of the solid organic matrix. They showed that the wear rates were directly related to the swelling ratios of the modified cartilage, and that the swelling ratios decreased with increased interaction between the cartilage constituents or cross-link density, and increased with an increased fixed charge density (which was assumed to be directly related to the glycosaminoglycan (GAG) concentration). However, loss of GAGs alone did not necessarily cause a reduction in the wear resistance of the cartilage. This was in contrast to earlier reports which postulated that the loss of GAGs in the early stages of osteoarthritis was correlative with the tissue becoming less wear resistant

(see section 2.7). Lipshitz et al suggested that the loss of GAGs in the diseased condition may be coincident with other changes occurring within the tissue, for example, a decrease in the intermolecular interaction density.

Several researchers investigated the effect of different load regimes on the wear of cartilage. Barnett (1956) concluded that intermittent compression was more damaging than sustained steady pressure, but still expressed surprise at the resistant nature of the cartilage surfaces even under unfavourable conditions. Radin and Paul (1971) found that oscillation alone produced little wear up to 500 hours, but that additional impact loading produced visible damage by as little as 12 hours and gross damage (cartilage worn through to the bone) by 192 hours. Radin et al (1973) subjected live rabbits to daily intervals of physiologically representative impulsive loading and concluded that increased calcification and stiffening of the joint occurred as a result of this high impact load. Later still Radin et al (1982) published data that indicated that artificial stiffening of the subchondral bone increased the wear of the cartilage, but that stiffening of the cartilage with gluteraldehyde decreased its wear. This was presumably due to increased cross-linking of the tissue constituents by the gluteraldehye, as suggested by Lipshitz et al (1980). It seems probable from these reports that impact loading of

the joint is potentially detrimental to the cartilage as a consequence of stiffening of the subchondral bone due to this particular loading regime. Lipshitz and Etheredge (1984) recorded alterations in the deformation of the cartilage as a result of altering the loading regime. They observed that after an uninterrupted compressive load, resumption of the sinusoidal motion allowed reswelling of the compressed, dehydrated regions of the cartilage. Linn (1967) observed a similar phenomenon, and this is discussed in more detail together with data from this research program in section 4.6.15.

Reports differed on the effectiveness of synovial fluid compared to a buffer or saline solution as a lubricant of cartilage under experimental conditions. Radin and Paul (1971) concluded that synovial fluid had no lubricating advantage over buffer at a load greater than 600lb. Radin et al (1982) again stated that there was no significant difference between the use of synovial fluid or buffer. However, Lipshitz, Stone and Glimcher (1978) found that synovial fluid reduced the wear rate between cartilage and formaldehyde-reacted cartilage by up to three times and increased the time at which a final constant wear rate was achieved. Owing to the different experimental conditions, Radin and Paul (1971) and Radin et al (1982) using whole joints and Lishitz et al (1978) using small flat cartilage plugs, it is difficult to draw any direct comparison between these two reports.

Only Simon (1971) compared the wear of cartilage in his experimental setup with that of other materials. He observed that the wear of cartilage was less than that of neoprene rubber, oak and balsa wood, but greater than that of several plastics. He also showed that the wear of fibrillated cartilage was greater than that of intact cartilage. However, in contrast, Radin et al (1982) showed that scarification (cutting) of the cartilage with a scalpel blade had little effect on the wear. This indicates that cuts in the cartilage alone do not directly influence the wear response of the cartilage, in the short term at least.

Two studies investigated the wear of whole joints by a rubbing action alone (Clarke et al, 1975, and Seirig and Gerath, 1975). Both made attempts to accelerate the normally slow, or negligible, wear rate of cartilage. Clarke et al (1975) ran post-mortem joints dry and Seirig and Gerath (1975) increased the load and speed of rubbing in a live rat joint in excess of physiological values. Both observed surface roughness of the loaded joints. Clarke et al (1975) stated that disruption of the cartilage in human hip joints occurred at sites where osteoarthritic damage had been detected clinically and progressed along orientated fibrillar layers perpendicular to the joint motion. This would seem to indicate that such damage progressed along the direction of the superficial collagen fibre network.

Finally, two studies investigated the effect of abrasive particles in the lubricant on cartilage wear. Lipshitz and Glimcher (1979) added calcium phosphate crystals at a concentration of about 1mg/ml to the lubricant. They observed that at a contact stress of 1.65MPa the cartilage was worn to the bone in two and a half hours. Sutro (1962) inserted fine carborundum particles into the knees of live rabbits. He concluded from his investigations that this resulted in an acute type of osteoarthritis with associated fibrillation and damage of the articular cartilage. He also observed that the granules had been engulfed by the synovial lining and concluded that this would reduce their abrasive qualities. No other investigations of the effect of particles in the joint have been reported.

4.3 THE WEAR APPARATUS

As already mentioned (section 4.1) a simple experimental system was designed in order to control and independently vary factors considered to be important in the wear response of articular cartilage. As such there was no attempt made to duplicate *in vivo* conditions although the nominal contact stress and relative speed used in standardised experiments were within physiological ranges.

4.3.1 The Apparatus Used to Measure the Wear and Creep of Articular Cartilage

A standard pin-on-disc wear machine was used to wear plugs of cartilage (attached to bone) against a stainless steel plate, or disc, with a surface roughness of R_a=2μm. A photograph and schematic diagram of the apparatus is given in figure 4.1. The plugs were mounted securely in a holder and loaded (over a range of 2-10kg) against a stainless steel counterface. The lubricant (12ml of quarter-strength Ringer's solution) was contained in a trough constructed on the counterface by a central Perspex disc and outer ring. The counterface, or disc, was rotated at a constant speed of 85mm/s for a period of 5 or 6 hours. After the wear period the lubricant was collected by syringe and the amount of cartilage debris measured either by a spectrophotometric assay of the sulphated glycosaminoglycans or by determination of the bound sulphate by ion chromatography. The vertical displacement of the cartilage during the wear and creep tests was measured with a D2/500A linear variable differential transducer (LVDT) attached to a chart recorder via an amplifier. The contact area between the counterface and the cartilage was measured before and after the wear test with pressure sensitive paper (Fuji Film Prescale; pressure range 5-25kg/cm^2) and image analysis (Joyce Loebl Magiscan 2A). The nominal contact stress was

calculated by dividing the load by the average contact area.

4.4 MATERIALS

4.4.1 Source of Cartilage Specimens

Samples of articular cartilage attached to subchondral bone were obtained from equine fetlock joints. The biochemical composition of equine articular cartilage is reported to be similar to that of other species (Vachon *et al*, 1990). The lower halves of equine legs were supplied by Potters Horse Slaughterers, Bristol. These were frozen within two hours of slaughter. The fetlock joints were dissected from the leg as necessary after thawing (figure 4.2). This was completed with extreme care so as not to damage the cartilage surface with the scalpel blade. Each joint was then examined and graded according to the degree of *in vivo* scratch marks observed on the articular surface (see table 4.1 and figure 4.58), labelled and refrozen. The synovial fluid from each joint was also labelled and frozen. The cartilage samples were standardised as far as possible by only using, in most experiments, the lateral side of fetlock joints taken from the front legs graded as normal by eye (i.e. there was no visible surface damage). The joints of the front and hind legs of quadrupeds are subjected to different forces (Schryver

et al, 1978). As it was considered necessary to standardise the material used as much as possible, fetlock joints from the front legs only were used. The force on the fetlock joint is about two times body weight (approximately 450kg) for the walk and four times body weight for the trot, (Bartel *et al*, 1978). However, variability within the material would still have existed between specimens as a result of uncontrolled factors such as the size, weight, age, breed and medical history of the animal.

Grade	Condition of Articular Surface
0	Normal, no scratch marks visible
1	Very fine scratch marks, just visible
2	Fine scratch marks, easily visible
3	Gross scratch marks

Table 4.1 *In vivo* scratch mark grades.

Cylindrical plugs (13mm in diameter) of articular cartilage attached to approximately 1cm of subchondral bone were cut from frozen equine fetlock joints (figure 4.3) in order to reduce any damaging effects to the cartilage due to overheating whilst drilling. Distilled water was used as a lubricant during the drilling procedure (figure 4.4) and this had the dual purpose of

acting as a coolant and maintaining the hydrated state of the cartilage. Pilot experiments with the friction apparatus showed the contact area expected for a maximum load of 10kg to be approximately 9mm for the curved surface of equine fetlock joints. The 13mm diameter cartilage plug, therefore, allowed a nominal 2mm wide zone between the contact area and the unconstrained edge of the cartilage. This reduced the possibility of increased wear owing to the unconstrained cartilage edge as observed in the report by Lipshitz and Glimcher (1979). Specimens graded as '0', with no evidence of any damage to the articular surface, from both the medial and the lateral side of the joint were used for experiments comparing cartilage damage to the nominal contact stress. Only those specimens cut from the lateral side of the joint and graded as '0' were used in the experiments to determine the effect of crystals on the articular cartilage. The graded scratch mark specimens were used in a separate set of experiments in which the total damage caused to the scratched specimens in the wear apparatus was compared to that of normal, undamaged articular cartilage.

4.4.2 Storage of the Specimens

All joints were initially stored at -18°C, as for the friction experiments. They were allowed to thaw overnight at room temperature after which the fetlock joint was carefully dissected. Each joint was then

refrozen prior to the cylindrical plugs being drilled from the joint. (Freezing of articular cartilage has been shown not to effect its mechanical properties, (Hori, 1973, Kempson, 1970, and Lipshitz and Glimcher, 1979)). After the wear tests the specimens were labelled and stored in formal saline (BDH; phosphate buffered, pH 7.3 @ 25°C) prior to any further preparation for electron microscopy.

4.4.3 Preparation of Synthetic Crystals

Four different types of synthetic crystal were prepared by Dr. P. Shellis, of the MRC Dental Group, Bristol, for the wear tests. These were supplied as monoclinic and triclinic calcium pyrophosphate dihydrate (m-CPPD and t-CPPD, respectively) and calcium hydroxyapatite (HA) (these are all crystals commonly found in the synovial fluid of human arthritic joints) and dicalcium phosphate dihydrate (DCPD) (brushite). All crystals were characterised by infra-red spectroscopy at Bristol, and subsequently by x-ray diffraction and scanning or transmission electron microscopy.

t-CPPD crystals grown in an acrylamide gel following the method of Harries *et al* (1983) (as for the friction experiments, see section 3.4.3) were used in pilot wear experiments (section 4.6.4). This technique, though, was time-consuming and only provided limited quantities of crystals (at most 100mg), even though they were reasonably pure and of a similar size to that found *in*

vivo (Dieppe and Calvert, 1983). Owing to the difficult nature of this method a second preparation technique that produced larger quantities of such crystals was used to complete the wear studies. The method of Mandel (1988) was followed where CPPD crystals were formed from a solution of calcium dihydrogen pyrophosphate (CDPP) crystals under varying conditions. t-CPPD crystals were formed from the growth of large orthorhombic calcium pyrophosphate tetrahydrate (o-CPPT) crystals, whereas m-CPPD crystals were prepared by the addition of magnesium chloride and calcium acetate to a heated solution of CDPP crystals. The HA crystals were prepared after the method of McDowell *et al* (1977), where phosphoric acid was added to a boiling suspension of calcium hydroxide under nitrogen. The preparation of the brushite crystals (DCPD) followed the method of Christoffersen and Christoffersen (1988), samples being precipitated from an acidic solution of a potassium phosphate buffer with the addition of calcium nitrate.

4.4.4. Characterisation of the Synthetic Crystals

The original aim was to use synthetic crystals that matched those crystals observed *in vivo* in size, morphology and composition, i.e. rhomboid or rod-like CPPD crystals of 0.5-10μm in length (Moradi, 1991), and tiny HA crystals approximately 10nm by 50nm but tending to aggregate into clumps up to 10μm in diameter, (Moradi, 1991). However, on further characterisation by

x-ray diffraction (see appendix) and electron microscopy, some of the supplied crystals were shown to have a different chemical composition, size and morphology to those expected. Because of time constraints and the difficulty of supplying relatively large quantities of pure synthetic crystals it was decided to investigate the effect of size and morphology of the crystal types already supplied on the damage to articular cartilage.

In particular, the two batches of t-CPPD had different compositions. One chemically pure sample (characterised by infra-red spectroscopy and x-ray diffraction) consisted of large rhomboid triclinic crystals approximately 100 by 100 microns when examined in the SEM. The second batch consisted mainly of large "star-bursts" (approximately 50 to 100 microns in diameter, see figure 4.5) which closely resembled the morphology of the orthorhombic calcium pyrophosphate tetrahydrate (o-CPPT) crystals described by Mandel *et al* (1988). Their composition was confirmed as o-CPPT by x-ray diffraction (see appendix, table A4). It was these o-CPPT crystals that were used in the wear tests on account of their very distinct morphology compared to the other two types of crystals used.

The m-CPPD crystals mainly consisted of rod-shaped m-CPPD crystals (approximately 1μm by 5μm) as expected when examined in the SEM, and by XRD (appendix, table A5) (see figure 4.6). These m-CPPD crystals were also used

in the wear tests. The HA crystals produced a characteristic XRD pattern (appendix, table A6) and the crystals were tiny rods, approximately 50 by 150nm when examined by TEM (see figure 4.7a). It should be noted here that it is well-documented (Dieppe & Calvert (1983) and Gatter & Schumacher (1991)) that individual HA crystals tend to aggregate into larger clumps up to several microns in diameter. This was confirmed by our SEM examinations (figure 4.7b). The DCPD crystals were unlike the typical wedge-shaped or arrow-headed morphologies described by (Gaucher *et al*, 1978). They were irregular in shape, but from SEM examination appeared to consist mostly of thin flat plates as described by Christoffersen and Christoffersen (1988) (see figure 4.8). XRD (see appendix, table A3) confirmed their composition to be that of dicalcium phosphate dihydrate. The CPPD crystals used in the pilot wear experiments ranged in size (figure 4.9) but were mainly rhomboid with an x-ray diffraction pattern typical of the triclinic form (see appendix, table A2) although additional lines were present.

4.5 EXPERIMENTAL METHOD

As discussed in section 4.2 standard methods used to measure the wear of typical engineering materials were not suitable for analysis of cartilage wear, primarily on account of the variable fluid content of this material. Quantitative chemical analysis of the

cartilage debris in the lubricant was, therefore, used in this study. Initially the concentration of sulphated glycosaminoglycans (GAGs) in the lubricant was measured by spectrophotometric analysis. However, owing to problems encountered with this assay most measurements of cartilage wear were carried out by quantitative analysis of the bound sulphate, in its inorganic form, with ion chromatography. An LVDT was also used to measure the deformation of the cartilage during the wear tests, although this subsequently proved unsuccessful as a sensitive measure of cartilage wear.

4.5.1 Method Used to Measure the Wear of Articular Cartilage

A standard pin-on-disc wear machine was used to wear plugs of cartilage attached to subchondral bone against a stainless steel plate. The cartilage specimens were carefully orientated in the specimen holder so that the central portion of the curved cartilage surface would be loaded against the disc (see figure 4.10). The plug was fixed securely in the specimen holder by means of three equi-spaced screws and the sample holder was screwed onto a platform for locating the weights. The cartilage was then allowed to rehydrate fully before the contact area was measured prior to the wear test.

Two complimentary pieces of pressure sensitive paper (one containing the dye and the other the developer)

were stuck together along one edge with Sellotape. This had the effect of protecting the paper from the wet cartilage surface. The total thickness of this "sandwich" was 0.28mm. The specimen holder was located in the end of the support arm and the pressure sensitive paper was placed on the counterface below the cartilage specimen. The cartilage surface was then gently brought into contact with the pressure sensitive paper, loaded and left for 20 seconds. (Lipshitz and Glimcher (1979) stated that normal, unworn cartilage almost instantaneously reached its equilibrium contact area.) The specimen was removed and the developed contact area labelled for future analysis. Three such areas were obtained before and after each wear test, allowing the average contact area to be calculated as below:

$$A(av) = (A_B(av) + A_A(av))/2$$

where A(av) is the total average contact area, $A_B(av)$ is the average contact area before the wear test and $A_A(av)$ is the average contact area after the wear test.

The specimen was then orientated so that wear of the cartilage surface would occur parallel to the direction of movement within the joint. The specimen holder was wedged in such a position that the cartilage was totally immersed in the lubricant but not touching the surface of the counterface. A dust cover was placed over the specimen holder and the counterface. The LVDT was located on the central axis of the weight and specimen

holder in order that the vertical movement of the cartilage specimen could be recorded as it was loaded and worn, (see figure 4.1). The cartilage was loaded and the zero position of the LVDT adjusted. The specimen holder was then wedged as before and the cartilage allowed to recover for a period of 5 minutes.

To initiate a wear test the counterface was rotated at a constant speed of 85mm/s (20 revolutions per minute) and the cartilage specimen was released gently into contact with the surface. The specimen was then loaded gently and the machine was allowed to run for either 5 or 6 hours. Unless stated otherwise, a standard load of 6kg was used. The vertical displacement of the cartilage was recorded throughout the test by the LVDT. When the allotted time had elapsed, the cartilage specimen was first removed from contact with the counterface and then the disc was stopped. The specimen was immediately placed in a fresh solution of quarter-stregth Ringer's solution and the cartilage allowed to recover. The lubricant was carefully removed from the trough by syringe, the volume recovered was recorded, and the lubricant sample was frozen prior to analysis. After allowing a 10 minute recovery period the contact area between the cartilage and the counterface was remeasured three times. The cartilage plug was stored in formal saline (BDH: phosphate buffered, pH 7.4 @ 25°C). The stainless steel counterface was removed, cleaned and sterilised with approximately 2.5% sodium hypochlorite

solution after each wear test. This method was followed for control experiments with no crystals in the lubricant and for further experiments with crystals present in the lubricant.

Three types of crystal were used in the pilot wear experiments: triclinic calcium pyrophosphate dihydrate (t-CPPD), dicalcium phosphate dihydrate (DCPD) (brushite) and calcium hydroxyapatite (HA), all at a concentration of 0.5mg/ml of Ringer's solution. Physiological concentrations of crystals recovered from synovial fluids tend to be in the range of 0.05-0.1mg/ml (Dieppe, 1988, and Swan, 1991). However, the highest concentration recorded by Swan (1991) was of the order of 2.5mg/ml ~~(0.25mg/ml)~~. The concentration of 0.5mg/ml used in the experiments discussed here was, therefore, considered to be high but not excessively so. In order to disperse the crystals throughout the lubricant during the wear test a plastic paddle was attached to the counter-balance arm (as seen in figure 4.11a). Apart from the presence of the paddle and the crystals the method followed exactly that with no crystals present in the lubricant.

For the final wear experiments to investigate the effect of crystals of different size and morphology in the lubricant, three types of crystal were again used: orthorhombic calcium pyrophosphate tetrahydrate (o-CPPT), monoclinic calcium pyrophosphate dihydrate (m-CPPD) and HA at a concentration of 0.5mg/ml of Ringer's

solution made up with crystal-saturated (o-CPPT, m-CPPD and HA respectively) distilled water to prevent dissolution of the crystals during the test procedure. Each crystal solution was dispersed ultrasonically for 20 minutes in a beaker of cold water (to reduce any heating effects) prior to the wear test in order to disperse the crystals evenly throughout the solution. Instead of the plastic paddle a flexible silicone rubber gate was used to disperse the crystals during the wear test (see figure 4.11b). This had an advantage over the rigid plastic paddle in that it could be carefully adjusted so that any crystals flung to the edge of the trough were collected and redispersed throughout the lubricant and that crystals flowing through the path of the contact area between the cartilage and the counterface were also dispersed.

4.5.2 Calibration of the LVDT

The LVDT was calibrated against a flat base micrometer screw gauge. The two were clamped securely, with the LVDT resting upon the base of the micrometer. The micrometer was then unscrewed and rescrewed by 5μm intervals for 50μm. The displacement of the LVDT was recorded on the chart recorder via the amplifier. An average was taken over all the 5μm intervals and a calibration figure of 15 divisions per 50μm was used for 50mV f.s.d. and therefore 37.5 divisions per 50μm for the more sensitive scale of 20mV f.s.d..

4.5.3 Method Used to Measure the Creep Deformation of Articular Cartilage

Prior to the wear test the creep of several of the cartilage specimens was measured for a period of 10 minutes. The cartilage specimen was loaded statically whilst the output from the LVDT was recorded on the chart recorder. After calibration the vertical deformation of the cartilage with time was calculated. The cartilage was then allowed to recover for a further 10 minutes prior to the wear test.

4.5.4 Estimation of Wear by Quantification of Sulphated Glycosaminoglycans

The assay originally used to measure the amount of cartilage debris in the lubricant was developed by Anthony Hollander, the Department of Pathology, the University of Bristol, and involved spectrophotometric measurement of the reaction between sulphated glycosaminoglycans (GAGs) (from the cartilage debris) and 1,9-dimethylmethylene blue (DMB) dye, (Farndale *et al*, 1986)

The wear suspensions were first treated with papain (Sigma: final concentration: 0.7 units/ml) in a digestion buffer of 20mM disodium hydrogen orthophosphate dihydrate, 1/3mM ethylene diamine tetra-acetic acid (EDTA) and 2mM dithiothreitol for two hours

at 65°C, in order to digest the large proteoglycan aggregates into smaller molecules of hyaluronic acid, core protein and sulphated GAGs, mainly chondroitin sulphate and keratan sulphate. 40µl samples of the digested wear lubricant were taken, to which 200µl of DMB dye was added. The DMB dye was prepared by dissolving 16mg of dye, 3.04g glycine and 2.37g NaCl in deionised water to a final volume of 1l. The pH was adjusted to 3.0 with concentrated hydrochloric acid. The reaction of DMB with the sulphated GAGs resulted in a quantitative colour change of the dye from blue to pink which was detected using a Titertrek Multiskan MCC/340 Mk II plate reader connected to an Amstrad PC2086 computer. The absorbance at 525nm was read immediately after the dye was added with a reference wavelength of 690nm. The change in absorbance of the samples was then calculated from the mean absorbance of six blank wells containing only dye and buffer. The concentrations of GAG in the wear samples was estimated from a standard curve derived from the reaction of DMB with whale chondroitin sulphate, (Sigma). Concentrations of 0, 6.25, 12.5, 25, 50, 75 and 100 µg/ml were used. The standard curve was linear. The sensitivity of this method was 5µg/ml. The mean of two samples was calculated for each wear solution. However, if the difference between the two measurements was greater than 15%, or if either value was less than the sensitivity level, then no value was recorded on the computer output.

4.5.5 Determination of a GAG Masking Effect by the Presence of Crystals in Solution

Initial pilot wear experiments completed with HA, CPPD and DCPD crystals in the lubricant indicated that crystals of CPPD and DCPD artificially lowered the GAG concentration measured by the spectrophotometric assay (section 4.6.2). These results led to a further series of experiments in order to ascertain why these two crystal types appeared to be masking the GAGs, (Hayes *et al*, 1991).

First a known concentration of whale chondroitin sulphate (Sigma) was measured in the spectrophotometric assay with and without crystals present in the solution. HA, CPPD and DCPD crystals were used at a concentration of 0.5mg/ml. Second, a wear lubricant with a known concentration of GAG was taken, crystals (0.5mg/ml) were added and the GAG concentration was remeasured. Finally, the same wear lubricant with a known concentration of GAG was taken and three other wear lubricants already containing the three types of crystal at 0.5mg/ml, HA, CPPD and DCPD, were added separately to the original solution with no crystals. The GAG concentration was again remeasured.

4.5.6 Estimation of Wear by Determination of Bound Sulphate by Ion Chromatography

As indicated in section 4.5.5 problems were encountered with the spectrophotometric assay and DMB dye when crystals were present in the lubricant. An alternative method of quantifying the amount of cartilage debris in the lubricant was therefore investigated. This involved analysis of the amount of inorganic sulphate (derived from the proteoglycans) present in the lubricant with ion chromatography.

The wear suspensions, of cartilage debris in quarter-strength Ringer's solution, were defrosted and stirred well with a magnetic flea placed in the glass vial containing the suspension to achieve, as far as possible, a uniform distribution of particles. 2ml samples were then taken by pipette whilst the suspensions were being stirred. For wear suspensions containing crystals, each 2ml sample was dialysed against 5% ethylene diamine tetra-acetic acid (EDTA), pH 7.4, at room temperature for two days in order to dissolve the crystals and then against quarter-strength Ringer's solution at room temperature for a further two days to remove the EDTA. The dialysis was carried out using 1/4" flat cellophane Visking tubing, with two changes of 2 litres each of the dialysing solution for each group of about ten 2ml samples. The samples were

recovered carefully and the tubing was then washed with 2ml of water and the washings added to the sample.

The preparation method for crystal-containing wear solutions then followed that of the wear solutions containing no crystals. Both types of suspension were held at -20°C overnight. The frozen solutions were freeze-dried in a Birchever freeze-dryer and then ashed at 600°C (Silvestri et al, 1982) for 15 minutes. The ash was suspended in 2ml of grade I (Purite) water and vortex mixed. The suspensions were left to stand for 5 minutes and vortex mixed again before being filtered through a 0.22μm cellulose nitrate membrane. The organically bound sulphate in the original samples was now in the form of inorganic sulphate dissolved in the filtrate and its concentration could be determined by any appropriate method. In this work, the sulphate concentration was determined by ion chromatography using a Dionex BioLC system.

25μl samples of the filtrate were taken and injected onto an AS4A column eluted with 1.8mM Na_2CO_3/1.7mM $NaHCO_3$ buffer. Detection was by suppressed conductimetry using 12.5mM H_2SO_4 as the regenerant in the micromembrane suppressor. Six standard sulphate concentrations were used: 1.5, 3.75, 7.5, 15, 22.5, and 30ppm for calibration. The standard curve was linear. The sensitivity of the ion chromatograph for measuring sulphate concentrations was 1.5ppm which compared well with that of the spectrophotometric assay, (1.5ppm is

equivalent to 1.5μg/ml compared to 5μg/ml sensitivity level for the spectrophotometric assay). For each wear suspension an additional sample was taken as described above, filtered directly through a 0.22μm cellulose nitrate membrane and the sulphate concentration of the filtrate determined. This was subtracted from the total sulphate content measured in the 25μl sample to correct for the free sulphate in the suspending medium. This final inorganic sulphate concentration for the 25μl sample, S, (in ppm) was then standardised in terms of the total volume of lubricant obtained from the wear test by using the following equation:

$$S_T = 40 \times S \times V,$$

where S_T was the total inorganic sulphate concentration in the wear lubricant in ppm, S was the inorganic sulphate concentration in the 25μl sample in ppm (40 x 25μl = 1ml), and V was the volume of the wear lubricant recovered in ml. The total inorganic sulphate concentration, S_T, was used in all results.

4.5.7 Preparation of Cartilage Wear Samples for Scanning Electron Microscopy

The preparation technique used (Cameron et al, 1976) attempted to minimise artefacts occurring at the cartilage surface. Prior to any preparation the excess bone on the cartilage plugs was removed leaving about 2mm of subchondral bone below the cartilage. These specimens were post-fixed in 2.5% gluteraldehyde in 0.2M

sodium cacodylate buffer, pH 7.4, overnight. Each operation was carried out at room temperature. Each specimen was then washed in 0.2M sodium cacodylate buffer for 30 minutes, with six changes of solution, followed by a further period of fixation in 2% osmium tetroxide in 0.2M sodium cacodylate buffer, pH 7.4, for one hour. The specimens were then washed in twelve changes of distilled water prior to being dehydrated through a graduated series of alcohol concentrations: 20, 40, 60, 70, 80, 90, 95 and 100% ethanol. The dehydration reagent was then replaced by 100% acetone (two 15 minute changes) before the cartilage specimens were critical point dried using carbon dioxide for two hours. Each specimen was then coated with gold prior to examination in a JEOL T330 scanning electron microscope. An accelerating voltage of 10kV was used and a tilt angle of 20° for enhanced contrast. The gold-coated specimens were also examined with a Wild stereoscan optical microscope.

4.5.8 Preparation of Cartilage Samples for Transmission Electron Microscopy

Full thickness slivers of cartilage were removed from the bone from which 1mm cubes were fixed in 2% gluteraldehyde in 0.1M sodium cacodylate buffer, pH 7.4 for four hours at room temperature. These were then washed in the same buffer and post-fixed in 1% osmium tetroxide in 0.1M sodium cacodylate buffer. After

further washes in buffer the tissue was dehydrated through a graduated series of ethanol (30, 50, 70, 90 and 100%) before being embedded in Taab resin. The embedded cartilage specimen was then sectioned on a Reichert OMU3 ultramicrotome and gold sections taken. These were double stained with uranyl acetate and lead citrate and viewed in a JEOL 1200EX transmission electron microscope.

4.6 RESULTS AND DISCUSSION

4.6.1 Control Tests to Ascertain Whether Proteoglycans or GAGs Were Leached From the Cartilage When Under Load

Concern was initially expressed that proteoglycans, or GAGs, may be leached from the cartilage when under load and so contribute to the concentration of GAGs measured in the wear lubricant. Experiments were, therefore, completed to determine the concentration of GAGs leaching into the lubricant. Plugs of cartilage submerged in Ringer's solution were statically loaded over a range of 2-10kg. 12ml of Ringer's solution was used, as for the wear tests, and the trough was constructed so that the depth of the Ringer's solution was the same as that for the wear test. These experiments were run in parallel with a wear test under the same conditions of load and time (5 hours). After the alloted time the lubricant was collected, as for the

wear test, frozen and later analysed using the spectrophotometric assay. A zero GAG concentration was recorded in all cases indicating that there was no significant leaching of the GAGs from the cartilage when under a range of static loads (2-10kg) for a period of 5 hours. The GAG concentrations measured for the wear lubricants were therefore assumed to be due totally to damage of the cartilage.

4.6.2 Pilot Wear Experiments With Crystals in the Lubricant and the Subsequent Determination of Masking of the Sulphated GAGs by the Crystals

Pilot experiments were completed to investigate the effect of crystals (HA, CPPD and DCPD) in the lubricant on the wear of articular cartilage. The spectrophotometric assay was used to measure the amount of cartilage debris in the lubricant by determining the concentration of sulphated GAGs present, (Hayes et al, 1991).

Table 4.2 shows the results obtained for these pilot experiments. It can be seen that there was no difference between the GAG concentration for the control with no crystals in the lubricant and the GAG concentration when HA crystals were present in the lubricant. However, the GAG concentrations for wear solutions containing CPPD or DCPD crystals were much lower than expected, particularly as there was visible damage to all the

articular surfaces after the wear test. It was expected, therefore, that damage of the cartilage with crystals present in the lubricant would be at least of the same order of magnitude as that with no crystals in the lubricant. These results were therefore considered to be specious.

	GAG conc. (mg/ml) Test 1	Test 2
No crystals	29.1	24.2
HA	30.4	23.4
CPPD	1.5	4.5
DCPD	3.3	2.0

Table 4.2 GAG concentrations from lubricants containing crystals obtained from pilot experiments.

A series of further experiments were completed in order to ascertain why the CPPD and DCPD crystals lowered the measured GAG concentration in the wear lubricant. Results from these experiments confirmed that all three crystal types, HA, CPPD and DCPD, produced this effect. The results are given in table 4.3. In the case where the crystals were added to a wear solution with a known concentration of GAG (23.2mg/ml) (table 4.3a) it is clearly shown that the GAG concentration was lowered by the presence of all three types of crystal. This was

also confirmed by the results given in table 4.3b (with the apparent exception of the HA crystals), where crystal-containing wear solutions were added to the original solution with no crystals.

	GAG conc. (mg/ml) (a)	(b)
No crystals	23.2	23.2
HA	13.6	26.1
CPPD	11.9	14.2
DCPD	15.2	13.0

Table 4.3 GAG concentrations for a wear lubricant with a) crystals only added, and b) a second wear lubricant containing crystals added.

The data were clearcut apart from the apparent anomaly of the HA crystal-containing solution (table 4.3b). For this series of experiments the volume of the solution was doubled by the addition of the crystal-containing wear lubricant, effectively reducing the concentration of crystals by half, from 0.5 to 0.25mg/ml. The lack of significant difference between the GAG concentration of the control solution with no crystals and that of the solution containing HA crystals may, therefore, be explained by a dose response, with twice the amount of cartilage debris per unit concentration of crystals present as previously. Individual HA crystals are known

to have a great affinity for aggregation in vivo and in vitro and would have been in the form of HA crystal aggregates when supplied in synthetic form. The crystals were not dispersed in an ultrasonic bath prior to these wear experiments and it could be imagined, therefore, that the surface area-to-volume ratio for the large HA aggregates was less than for individual CPPD and DCPD crystals, hence, fewer GAGs would be able to bind to the available HA surfaces. More free GAGs would, therefore, be detected by the assay in the case of the HA crystals. In general, however, the data given in tables 4.2 and 4.3 indicate that all three types of crystal were in some way masking the sulphated GAGs and preventing their detection in the spectrophotometric assay when DMB was used as the dye reagent.

It is interesting to note that results obtained with solutions of commercial whale chondroitin sulphate and the three different crystals showed that there was no significant difference in the sulphated GAG concentrations with and without crystals present. This indicated that the presence of the crystals in the chondroitin sulphate solution did not have the same effect as those in the wear lubricant in masking the GAGs.

In order to ensure that the crystal solutions alone were not interfering with the DMB dye and thus initiating an artificial colour change, three crystal solutions containing 0.5mg/ml Ringer's of HA, CPPD and DCPD

crystals were left at room temperature for six hours and then frozen prior to being assayed, as for the wear lubricants. This was to ensure that approximately the same amount of dissolution of the crystals would occur as in the wear lubricants. All three solutions gave zero readings in the spectrophotometric assay indicating that they did not interfere with the DMB dye, and were not the cause of the specious results given above.

In summary, crystals present in the wear lubricant appeared to mask the GAGs from detection by spectrophotometric analysis with DMB dye, but the presence of crystals in a solution of chondroitin sulphate did not have this effect. It is well documented that proteoglycan aggregates, monomers and GAGs bind to the surface of HA crystals, in particular, thus causing an inhibitory effect on crystal formation and growth, (Embury et al, 1979, Chen et al, 1984, Chen & Boskey, 1985, Hunter et al, 1985, Tenenbaum & Hunter, 1987, Hunter, 1987, Dziewiatkowski, 1987, and Hunter et al, 1987). Hunter et al (1987) also provided evidence that chondroitin sulphate inhibited the formation of CPPD crystals in vitro by apparent binding of the Ca^{2+} ions. Factors which are known to be important in the inhibition of crystal growth include a) the electrostatic interaction between the divalent cations and the anionic groups of the proteoglycans (Embury et al, 1979, and Hunter, 1987); b) the stereochemical properties of each interacting surface (Addadi and

Weiner, 1985); c) the presence of sulphate groups within the macromolecule (Chen & Boskey, 1985 and Hunter et al, 1985); d) the charge density of the macromolecule (Chen and Boskey, 1985, and Chen et al, 1984) and hence the hydrodynamic size of the macromolecule; and e) the status (i.e. proteoglycan aggregate, monomer or GAG) of the macromolecule (Chen et al, 1984, Hunter, 1987, Dziewiatkowski, 1987, and Hunter et al, 1985). It was assumed that these factors were, therefore, also important in the binding mechanism of the proteoglycan, or GAG, to a crystal. A variety of cartilage proteins have also been shown to have calcium-binding properties (Kozin & McCarty, 1976, Dieppe & Calvert, 1983, and Hunter, 1987). Indeed, the use of calcium hydroxyapatite in chromatographic columns is related to characteristic adsorption behaviour of individual proteins and peptides onto this adsorbent (Moreno et al, 1984).

The exact mechanism by which the GAGs in the wear lubricant interacted with the crystals could not be specified. However, in the light of the experimental results given in table 4.3 and documented evidence described above, it cannot be disputed that some such interaction took place. The fact that the detection of commercial chondroitin sulphate was not influenced by the presence of crystals suggested that the protein attached to the GAG had an important role to play in the binding of the crystals to these macromolecules.

4.6.3 Repeatability of the Spectrophotometric Assay

In order to evaluate the error associated with measuring the concentration of cartilage debris with the spectrophotometric assay twenty aliquots of one lubricant (without crystals) were taken and the concentration of sulphated GAGs present was simultaneously measured in each aliquot. The mean GAG concentration recorded was 12.5mg/ml with a standard error of 0.22mg/ml (equivalent to approximately 2%). This calculated error was considered to be satisfactorily low.

4.6.4 Increase in GAG Concentration With Time When Measured With the Spectrophotometric Assay

In order to ascertain the error associated with the plate reader in measuring the concentration of GAGs in the wear solutions one plate prepared with fifteen different solutions was read three times in succession. The results for the eight solutions that registered a GAG concentration higher than the sensitivity level of the calibrated system are given in table 4.4 and illustrated in figure 4.12.

Specimen	GAG Concentration (mg/ml) Test 1	Test 2	Test 3
1	8.86	9.47	10.31
2	15.70	16.67	18.86
3	18.64	19.43	18.43
4	43.01	45.89	46.51
5	29.78	32.94	34.41
6	20.69	22.94	23.95
7	21.48	26.01	26.77
8	16.78	19.09	19.47

Table 4.4. The GAG concentrations (mg/ml) measured in three consecutive tests for eight wear solutions.

In all but one case the GAG concentration gradually increased throughout the consecutive tests and did not vary randomly. This suggested that the digestion of the proteoglycans with the papain was not complete after two hours and was continuing throughout the time taken to carry out the consecutive tests. It was thus concluded that the enzymatic digestion of the cartilage debris required halting at the end of the two hour digestion period either by reducing the temperature by placing the solutions on ice, or with a biochemical inhibitor to papain. These results also indicated that two hours was insufficient for total digestion of the cartilage debris, with larger molecules only being partially digested. It was thought that this could have important implications for further results if different crystal types produced different sized particles of cartilage debris.

4.6.5 Correlation Between the Spectrophotometric Assay and the Ion Chromatography

Owing to problems encountered with the spectrophotometric assay and the presence of crystals in the wear solution an alternative method of measuring the concentration of cartilage debris was investigated. This involved the analysis of the concentration of inorganic sulphate in the wear solution by ion chromatography, as described in section 4.5.5.

Control experiments measuring the wear of articular cartilage without crystals present in the lubricant were already in progress, and results had been obtained with the spectrophotometric assay. It was, therefore, of some importance to ascertain whether there was any correlation between the wear measured by the assay and that measured by ion chromatography in order to ascertain whether the results of these initial experiments would be valid. Wear solutions for normal cartilage under a range of loads (from 2 to 10kg) had already been analysed with the spectrophotometric assay and then stored at -20°C. These were remeasured by ion chromatography and the two sets of results correlated. The results, shown in figure 4.13, had a linear correlation coefficient of 0.88 which was significant at the 0.1% level. It was, therefore, concluded that both of these independent techniques were suitable for

measuring the concentration of cartilage debris in a solution providing that no crystals were present.

Problems were also encountered with ion chromatography when crystals were present in the solution in that the measured concentration of inorganic sulphate ions was again artificially lowered, as for the spectrophotometric assay. It was suggested that any free calcium ions present in the solution may have bound to the sulphate ions released from the cartilage debris, thus forming bound calcium sulphate which was not then detected by the ion chromatograph. This problem was overcome by dialysing the wear solutions against EDTA to dissolve the crystals, as described in section 4.5.5.

Ion chromatography of the inorganic sulphate content was, therefore, established as a technique to measure the concentration of cartilage debris in solutions with and without crystals present. This technique was used in all subsequent experiments.

4.6.6 Repeatability of the Ion Chromatography

In order to ascertain the error associated with measuring the concentration of inorganic sulphate present with ion chromatography, ten 25ml aliquots of two lubricants (one containing crystals and the other without) were consecutively measured in the ion chromatograph. The mean inorganic sulphate concentration for the lubricant without crystals was 1.87ppm with a

standard error of 0.1ppm (0.6%). The mean inorganic sulphate concentration for the lubricant containing crystals was 8.87ppm with a standard error of 0.05ppm (0.6%). This error compared favourably with that obtained with the spectrophotometric assay (of 2%), although unlike this assay each 25ml aliquot was taken from the same 2ml sample and not from different samples of the original lubricant. The total error associated with one 25ml aliquot would, therefore, be expected to be higher than the 0.6% calculated here, which represents the error associated only with the ion chromatograph.

4.6.7 The Wear of Articular Cartilage Over a Range of Normal Loads

The wear, or damage, of articular cartilage for loads ranging from 2-10kg was initially measured by analysing the sulphated GAG concentration in the wear lubricants with the spectrophotometric assay. The stored lubricants were then remeasured by ion chromatography. These results were subsequently expanded when further tests were completed with the ion chromatograph. The contact areas for each specimen were measured with pressure sensitive paper and hence the nominal contact stresses were calculated by dividing the applied load by the total average contact area (as shown in section 4.5.1).

Load Dependence of Wear

The combined ion chromatography results for this range of loads are illustrated in figure 4.14. Figure 4.14a gives the total inorganic sulphate concentration measured for the 5 hour test period in parts per million (ppm) against the applied load. The mean and one standard error are shown for each 2kg load interval. An initial analysis of these results suggested an approximately linear relationship between the amount of cartilage debris measured in the lubricant and the load. This trend basically fitted the empirical law of wear for standard engineering materials which states that wear generally increases with load (Bowden and Tabor, 1974). However, further analysis using Student's t-test showed that there was no significant difference between the total amount of cartilage debris (inorganic sulphate content) at 2 and 4kg, and that there was no significant difference between the amount of debris at 6, 8 and 10kg, but that there was a significant difference between the total amount of debris at 4 and 6kg, at the 2% level. Hence there appeared to be two distinct groups of data represented in figure 4.14a, those specimens worn at a load of 4kg or less, and those worn at a load of 6kg or more with this latter group being worn significantly more than those at the lower loads. It may be implied from these results that above some load between 4 and 6kg the resistance of the cartilage to damage was compromised. However, once this wear

resistant barrier was broken the cartilage wore again at a relatively constant rate (up to a load of 10kg, at least).

Dependence of Wear on Contact Stress

Figure 4.14b shows the total amount of cartilage debris in the lubricant (measured as the total inorganic sulphate content for the 5 hour test period in ppm) against the nominal contact stress for each specimen. The mean and one standard error are again shown. However, as the sample sizes were small (less than six in all cases) an estimated standard deviation, equal to the range divided by a constant (dependent on the sample size), d_n, was used to calculate the standard error, (Brownlee, 1957). Although the individual data points were widely dispersed these results again suggested an increased trend between the amount of cartilage debris measured and the nominal contact stress. It was again found from Student's t-test that there was a significant difference at the 0.2% level between the total inorganic sulphate concentration measured for nominal contact stresses above 1.75MPa (2399ppm) and that measured for nominal contact stresses below 1.5MPa (1202ppm). These data also suggested, therefore, that there was a specific load, or stress, at which the damage resistance of the cartilage was compromised.

It was also interesting to note that, unlike many standard engineering materials, the wear of cartilage

(measured by analysis of the concentration of debris in the lubricant) was dependent on the nominal contact stress applied. These data were supported by results obtained by Lipshitz and Glimcher (1979) which indicated that the wear rate of articular cartilage was dependent on the pressure applied. This indicated that the apparent contact area was an important factor in determining the wear properties of articular cartilage, and that the asperity-asperity contact theory generally applied to hard materials was not valid in the case of relatively soft, deformable articular cartilage being worn against a hard counterface.

Errors Associated With Measuring the Contact Area

The wide dispersion of the data points was partially due to the variable nature of the material tested, but must also be a function of the errors associated with measuring the applied stress. As stated above, only the nominal contact stress was measured. This was achieved by measuring the contact area with pressure sensitive paper, and then dividing by the applied load. However, there were several errors associated with the measurement of the contact area in this way. First, the area in contact with the paper was dependent on the time that the load was applied, (as shown in figure 4.15). Lipshitz and Glimcher (1979) stated that the equilibrium contact area would be achieved almost instantaneously upon loading the cartilage. This was not found to be the

case, but on further investigation 20s was considered to be a reasonable compromise between efficient use of time and accuracy. On reflection it may have been better to allow one minute for the cartilage to deform before measuring the final contact area. Figure 4.15 indicated that after one minute approximately 96% of the total contact area would be recorded (assuming that 100% is in contact within 5 minutes), compared to 86% after 20s. However, the time taken to measure the contact area, at 20s, was consistent throughout all the experiments.

Second, the contact area had to be measured with the cartilage dried from excess fluid, as this affected the dye in the pressure sensitive paper. It was possible that this affected the frictional properties of the cartilage compared to the situation during the wear test when the cartilage would have been immersed in liquid. However, to possibly counteract this the cartilage was deformed against a relatively smooth shiny surface, provided by the layer of Sellotape sticking the two pieces of pressure sensitive paper together, compared to the relatively rough metal plate, and alsc liquid would have been squeezed from the cartilage upon loading to provide some lubrication to counteract any frictional effects.

Third, the random error, associated with all measuring systems, was taken account of by measuring the contact area three times for each specimen, before and after the wear test, each with a mean and associated standard

error. The mean coefficient of variation calculated for 25 samples was 3.1% (which was itself associated with a standard error of 0.3%). This provided a measure of the random error associated with measurement of the contact area and was considered to be comparatively low.

Summary

In consequence of these errors it was difficult to investigate the data on a point-by-point basis, although general trends were observed. First, the data were divided into two separate groups, that worn at a stress below 1.5MPa and that worn at a stress above 1.75MPa. The existence of a stress level (between 1.5 and 1.75MPa) at which there was, relatively, a sudden change in the wear behaviour of cartilage indicated that there could exist a wear resistant layer which at a nominal contact stress below 1.5MPa remained relatively intact under the test conditions described here and had the effect of protecting the cartilage, thus keeping the total damage to a minimum. However, at a stress above 1.75MPa it could be imagined that this resistant layer broke down thus increasing the associated damage to the cartilage. However, it was not clear from these results whether the total wear (measured by the concentration of inorganic sulphate in the lubricant) would gradually increase with an increased applied load or whether, as for the lower stresses, the total wear would remain relatively constant over a range of loads.

Unfortunately, 10kg was the maximum load that could be applied in the apparatus described in section 4.3.1 and so this could not be investigated.

Lipshitz and Glimcher (1979) state that when wearing articular cartilage from the medial femoral condyles of adult steers against a stainless steel plate with ridges running in the wear direction of about 0.5mm deep, 1.1mm wide and 1-2.6mm apart, there was an abrupt change in the wear rate-pressure relation at around 2.07 x 107 dyn cm-2 (2.07 MPa) after which a constant wear rate was attained. This value is a little higher than that estimated from the results shown in figure 4.14, for equine articular cartilage worn against a stainless steel plate of surface roughness, Ra, equal to approximately 2μm. However, the general behaviour appeared similar.

4.6.8 The Wear of Articular Cartilage With and Without Crystals Present in the Lubricant

Ion chromatography was used to measure the wear of normal equine articular cartilage without crystals and with three different types of crystal present in the lubricant. Control tests with no crystals present in the lubricant (Ringer's solution) were completed for eleven specimens and the effect of each crystal type was tested on three specimens. Three different types of crystal were used: orthorhombic calcium pyrophosphate

tetrahydrate (o-CPPT), monoclinic calcium pyrophosphate dihydrate (m-CPPD) and calcium hydroxyapatite (HA).

Figure 4.16 shows the wear of the cartilage (measured as the inorganic sulphate content present in the lubricant in ppm) for the controls (with no crystals present) and for the three crystal types. The mean total inorganic sulphate concentration for the six hour test is shown together with the standard error for each case. There was no significant difference between the total amount of wear measured for each crystal type (as indicated by Student's t-test) but there was a significant difference at the 0.2% level between the total mean wear for the controls (1988ppm) and that for the crystals (mean of 3913ppm).

It was clear from these results that the presence of crystals had a marked effect on the amount of sulphated GAGs released into the lubricant with approximately twice the concentration in the case of the crystals compared to that of the controls. However, it was interesting to note that there was no significant difference between the concentration of sulphated GAGs measured in the lubricant for the different types of crystal. This, at first, was surprising as the morphology and size differences between the individual crystal types were quite marked: the o-CPPT crystals were large (50-100μm in diameter) with many sharp, pointed facets, the m-CPPD crystals were small (approximately 1μm by 5μm) and rod-shaped, and the HA

crystals were tiny rods (50nm by 150nm) but known to aggregate into larger clumps up to about 10μm, (Dieppe & Calvert (1983) and Moradi (1991)). Further work with scanning electron microscopy (see section 4.6.17) indicated, however, that the type of damage caused by each crystal type was unique even though, as suggested here, the amount of cartilage removed was not significantly different.

4.6.9 Measurement of the Vertical Displacement of the Cartilage During the Wear Test

An LVDT was used to measure the vertical displacement of the cartilage during the wear test, a trace of which was recorded on a chart recorder. It was originally hoped that this information would provide an independent measure of the damage, or wear, of the cartilage which could then be correlated with results obtained with the ion chromatograph.

It was anticipated that as cartilage behaves as a viscoelastic material it would exhibit creep, a measure of which would have to be obtained in order that the wear (taken here as the vertical thickness of cartilage removed) alone could be calculated from the LVDT measurements. The creep of a normal cartilage specimen was recorded with the LVDT for a period of five minutes with the intention of subtracting this curve from that recorded during the wear test in order to obtain the

wear curve alone. However, it was found that the creep curve exhibited a greater vertical displacement than the supposed wear plus creep curve recorded by the LVDT during the wear test, (see figure 4.17). This phenomenon was investigated further and will be discussed later in section 4.6.15.

Determination of the Creep Component

In order to interpret the LVDT data a measure of the creep of each specimen was still required in order that the wear alone could be calculated. LVDT plots for four specimens were recorded over a period of ten or twenty minutes, and a log/log plot of the data was made to ascertain whether there was a significant change in the slope of the curve at a particular time. It was assumed that this would indicate a change in the behaviour of the cartilage as the displacement due to creep equilibriated with the displacement due to wear. In three out of four cases, there was a significant change in the slope of the curve at a logtime corresponding approximately to three minutes. A typical example is given in figure 4.18 where the logarithm of the vertical displacement recorded with the LVDT is plotted against the logtime. In the fourth case the time at which the behaviour changed significantly was five minutes. It was assumed that the majority of the creep response of the cartilage, as recorded with the LVDT, had ceased after the first five minutes of a wear test in order to ensure

that values assumed to be mainly due to creep were well outside the transition period. The vertical displacement at five minutes was therefore subtracted from the rest of the curve to produce a plot of the displacement due to damage alone, as seen in figure 4.19.

Errors Associated With the LVDT

There were several errors associated with the measurement of the vertical displacement of the cartilage with an LVDT, the largest being the difficulty of making the start of the trace coincide exactly with the initial loading of the specimen. This meant that the magnitude of the initial creep phase recorded varied from trace to trace. Subtraction of the creep after five minutes from the rest of the trace was therefore an approximate means of standardisation for the remaining wear curve. Other errors were associated with the vertical alignment of the LVDT, and a wobble in the counterface as it rotated so that the trace recorded from the LVDT was a band, approximately 50μm wide, rather than a single line. An example is given in figure 4.20. In order to obtain values for the vertical displacement from this trace the centre point of the band was located and this point was taken as the displacement for the specific time. All of these errors would have contributed to the difference in the logtime recorded in the fourth case discussed above.

LVDT Measurements With and Without Crystals Present in the Lubricant

Figures 4.21a-d illustrate the mean LVDT plots after the subtraction of the five minute creep component for no crystals and for the three crystal types present in the lubricant. Figure 4.21a (no crystals) is the mean plot of five separate curves, figure 4.21b (o-CPPT crystals) is the mean of four curves, and figure 4.21c (m-CPPD crystals) is the mean of three curves as is figure 4.21d (HA crystals). The standard errors for each mean point plotted are shown. Each curve has been plotted on the same scale axis for comparison. It can be seen from these graphs, although more clearly illustrated in figure 4.22, that the LVDT plots for all four cases, with and without crystals, did not show any significant differences. These data, therefore, appeared to contradict the evidence given in section 4.6.8 that the presence of crystals in the lubricant led to twice as much wear, in the form of cartilage debris, than when no crystals were present.

4.6.10 Comparison of Theoretical Predictions of the Thickness of Cartilage Removed With Experimental Measurements

Some concern was expressed that the resolution of the LVDT may not have been sufficient to detect the small vertical displacements associated with the cartilage

being worn away, and hence the result discussed in section 4.6.9, that there was no difference in the vertical displacement with and without crystals, may not have been valid.

Experiments had been completed (see section 4.6.12) that provided data of the contact area for one specimen for every hour of running of a wear test. These data were used in conjunction with a scale drawing and some simple arithmetic to ascertain the approximate theoretical thickness of cartilage that would be worn away. A radius of curvature of the joint of 15mm was used, and it was assumed that the contact areas were circular to facilitate the calculations. The scale drawing, illustrated in figure 4.23, gives a clear idea of the relative thicknesses of cartilage removed (as measured with the LVDT). Figure 4.24a shows the geometrical factors used in the model to calculate the theoretical thickness of cartilage worn away. From Pythagorus's theorem:

$$R^2 = h^2 + r^2, \text{ therefore, } h = (R^2 - r^2)^{1/2},$$

where R is the radius of curvature of the joint (15mm), and r is the radius of the circular contact area. The differences between the heights, h_4 and h_3 for example (see figure 4.24a), gave the thickness of cartilage worn away. The results are given in table 4.5.

Time (hours)	Areas (mm^2)	Radius, r (mm)	Thickness of cartilage worn away (μm)
0	18.7	2.4	
1	29.3	3.1	131
2	33.9	3.3	44
3	41.8	3.6	70
4	47.0	3.9	78

Table 4.5 Theoretical predictions of the thickness of cartilage worn away for measured contact areas.

The theoretical predictions are plotted against the values measured with the LVDT for the vertical displacement of the cartilage (discussed in detail in section 4.6.12) in figure 4.25. Considering the errors involved, first, in measuring the vertical displacement with the LVDT and second in calculating the theoretical thickness of cartilage worn away, in the use of the basic assumptions described above, the experimental measurements were considered similar and they are certainly of the same order of magnitude. Therefore, returning to the original objective of this particular investigation, it was concluded that the resolution and accuracy of the LVDT were sufficient to measure the small overall vertical displacements associated with the wear of cartilage in this experimental setup. The result described in section 4.6.9, that there was no significant difference between the overall average vertical displacement measured with the LVDT with and

without crystals, was, therefore, considered to be valid.

However, the total theoretical prediction of the thickness of cartilage removed (approximately 320μm) was much greater than that measured experimentally with the LVDT (maximum 125μm). The theoretical predictions were, therefore, exaggerated compared to experimental results. This was probably due to some of the assumptions used in the calculations where a real oval-shaped contact area was assumed to be circular. Owing to the geometry of the joint (being cylindrical rather than sperical) the radius of the assumed circular contact area, r, actually corresponded to the minor axis, b, of the oval-shaped contact area (see figure 4.24b). The calculated radius of curvature, r, (from the assumed circular contact area) was larger than the minor axis, b, and as is clearly shown in figure 4.24b the calculated thickness of material removed will, therefore, be greater than that observed experimentally.

4.6.11 Comparison of the Mean Contact Areas Before and After the Wear Test

The contact areas between the cartilage and the stainless steel counterface were routinely measured with pressure sensitive paper before and after the cartilage was worn and the mean contact area calculated for each case. The ratio of the mean contact area after the wear

test to the mean contact area before, i.e. $A_A(av)/A_B(av)$, was calculated for each experiment completed with and without crystals in the lubricant. There was no significant difference between the mean ratio $A_A(av)/A_B(av)$ for crystals and that for no crystals in the lubricant, the mean value of $A_A(av)/A_B(av)$ being 1.82 in both cases (with sample sizes of twelve). The standard error for the controls with no crystals was 0.09 and that for crystals was 0.08.

Together with the data obtained with the LVDT, where it was concluded that the thickness of cartilage removed was the same for controls and crystal-containing lubricants, the above result confirmed that the overall volume of cartilage removed was constant, being independent of whether there were any crystals or not in the lubricant. Considering results obtained with ion chromatography where a significant difference was observed in the concentration of inorganic sulphate measured in the lubricant with and without crystals, it was, therefore, concluded that the physical measure of volume change, or thickness of cartilage removed (an LVDT and pressure sensitive paper being used to measure the vertical displacement and contact area, respectively), was not a sufficiently sensitive method to use as a quantitative measure of cartilage damage, or wear. Indeed, as Lipshitz *et al* (1975) state, dimensional changes of the tissue as it is being worn away may not parallel those of wear because the volume

of cartilage is a function of its fluid content which varies with the appiled pressure. Chemical analysis of the amount of wear debris produced, such as with ion chromatography, proved to be a more sensitive measure of the damage incurred to the cartilage.

Summary

The data obtained with the ion chromatography, therefore, indicated that the presence of crystals in the lubricant caused approximately twice as much damage to the cartilage as when no crystals were present. However, data obtained with the LVDT and by measuring the contact areas before and after the wear tests indicated that there was no difference between the overall volume of cartilage removed with and without crystals present. Two possibilities exist to explain this anomaly. First, the increased concentration of inorganic sulphate (derived from the cartilage debris) measured when crystals were present in the lubricant could have been due to deeper surface scratches caused by the crystals, but not detected by the LVDT. Figure 4.26 illustrates how it could be possible for such scratches to remain undetected by the LVDT. Second, the additional debris measured by ion chromatography could have come from cartilage surrounding the contact area but still immersed in the lubricant, i.e. not in contact with the counterface. Any removal of material from this

area would not have been detected by any vertical displacement of the LVDT.

4.6.12 The Wear of Articular Cartilage With Time Measured With the LVDT and Ion Chromatography

During the wear tests the cartilage specimen was worn for the full five or six hours before the lubricant and the specimen were recovered for later analysis. However, no information could be obtained from these experiments with respect to the behaviour of the cartilage with time. A short investigation of the wear response of cartilage with time was therefore carried out by means of LVDT and ion chromatograph measurements and also by measurements of the contact areas at hourly intervals.

A specimen of normal equine articular cartilage was worn under the standard conditions described in section 4.5.1 for an hour followed by a twenty minute recovery time to enable the lubricant to be collected and to allow the cartilage to reimbibe fluid. This necessitated the removal of the LVDT, and so a common base line was assumed in the presentation of these data, shown in figure 4.27a.

The first trend to note is the decreasing displacement measured with time. The displacement here included the contribution from creep (i.e. the total vertical displacement) and is more graphically illustrated in figure 4.27b. The contribution of the creep (taken after

five minutes) is also shown in figure 4.27b. The creep of the cartilage as it was gradually worn decreased rapidly at first but then apppeared to stabilise at a constant value of approximately 20μm after three hours. When the creep component at five minutes was subtracted from the total displacement the decreasing trend of displacement with time more or less disappeared (see figure 4.27c). However, the measurement of displacement only gave an indication of the amount of material removed in the vertical direction, and took no account of the volume of material removed.

The concentration of cartilage debris measured with the ion chromatograph with time is given in figure 4.28. Apart from the second hourly interval the concentration of cartilage debris measured in the lubricant tended to gradually increase with time. The contact area (figure 4.29) also increased with time as would be expected. The five hourly result is obviously an anomaly, and must be assumed to have fallen victim to the range of errors associated with measuring the contact area as discussed in section 4.6.7.

When discussing these results it must be remembered that this experiment was conducted on one specimen only, and hence it would be unwise to draw any firm conclusions. However, some trends can be observed. The increase in the total inorganic sulphate content measured by ion chromatography would tend to indicate that either the wear rate of the cartilage increased with time (assuming

that the concentration of proteoglycans was relatively constant throughout the depth of the cartilage), or that the concentration of GAGs in the cartilage increased with depth in the cartilage (assuming that the volume of material worn away remained fairly constant), or that a combination of both of these factors was important.

Meachim and Stockwell (1979) presented evidence from a survey of the literature to suggest that the concentrations of both chondroitin sulphate and keratan sulphate increased gradually with the depth of cartilage from 10 to 40% of the total cartilage thickness (measured from the articular surface). These data are illustrated in figure 4.30. This information was supported by Lipshitz *et al* (1980). The data recorded by Meachim and Stockwell (1979) was for normal *human* articular cartilage where the superficial zone is documented as being approximately 5-20% of the total cartilage thickness (Meachim and Stockwell, 1979). However, results obtained for equine articular cartilage (see section 4.6.16) suggested that the superficial zone for articular cartilage from the fetlock joint was only about 8μm from a total thickness of approximately 1mm (i.e. 1%). The superficial zone is known to contain a majority of collagen fibres and very few matrix molecules (i.e. GAGs) This is presumably reflected in the low GAG concentration in the top 10% of the cartilage as shown by Meachim and Stockwell (1979). It is assumed here that owing to the relatively thinner

superficial zone in the equine fetlock cartilage the relative thickness of cartilage with very low GAG concentrations will be significantly less than 10%. The maximum final vertical displacement measured experimentally with the LVDT (indicating the thickness of cartilage removed) was 125μm which represented about 10% of the total cartilage thickness. Hence, it was conceivable that the concentration of GAGs varied with the depth of cartilage removed in this region.

The data, illustrated in figure 4.27c, indicated that the thickness of material removed remained approximately constant, with maybe a slight decrease after three hours. This indicated that, at least during the first two or three hours, the volume of cartilage removed increased (as a result of an increase in the contact area from one hourly period to the next), but that after three hours the volume of cartilage removed could be expected to decrease, relatively, or perhaps remain constant. It therefore appears likely that both an increase in the GAG concentration with depth of cartilage removed and an increase in volume of cartilage removed were important factors in the increase of the concentration of inorganic sulphate recorded with time, but that after three hours of testing the increase in inorganic sulphate measured in the lubricant was due to an increase of sulphated GAGs with depth of cartilage. This corresponded to an approximate vertical displacement of about 80μm.

4.6.13 Wear of Articular Cartilage Scratched *In Vivo*

After the fetlock joints were dissected each was graded according to the severity of visible parallel scratch marks on the articular surface (as described in section 4.4.1) where grade 0 indicated no visible damage to the articular surface and grade 3 indicated the presence of gross scratch marks on the articular surface. Such "track marks" have been reported previously by Freeman & Meachim (1979). These graded specimens were tested in the wear apparatus as previously described in section 4.5.1. The amount of cartilage debris in the lubricant was measured with ion chromatography (by analysis of the inorganic sulphate concentration) and the overall vertical displacement of the cartilage during the test was measured with the LVDT.

The control data for the grade 0 specimens were the same as those used for the experiments conducted to investigate the wear of cartilage with and without crystals in the lubricant. Eleven grade 0 specimens were used and five specimens for each grade, 1,2 and 3 were also tested. The results from the ion chromatography, showing the concentration of inorganic sulphate from the cartilage debris in the lubricant for each of these grades, are shown in figure 4.31. The means and the standard errors for each group are given.

There was no significant difference between any of the scratch grades at the 5% level, although the mean

inorganic sulphate content for the grade 2 specimens at 4469ppm appeared at first sight to be significantly larger than the collective mean of the other three grades at 1884ppm. It was also interesting to note the apparent wide range of values recorded from the ion chromatography measurements for the grade 2 specimens. These are given in table 4.6. However, when the coefficient of variation was compared across the three grades it was observed that it was similar for all grades, hence the lack in significant difference.

Specimen	Total inorganic sulphate content (ppm)		
	Grade 1	Grade 2	Grade 3
1	1182	1348	2552
2	1890	1837	1529
3	2721	6504	92
4	3410	8244	1179
5	1125	4412	2647
Mean	2066	4469	1600
Stan. error	443	1324	472
Coeff. of variation	21%	30%	30%

Table 4.6 The total inorganic sulphate concentrations measured with the ion chromatograph for each scratch grade.

One possible explanation of the wide range of inorganic sulphate concentrations measured for the grade 2 specimens was the fact that the grading system was

necessarily coarse, being based on a lumped parameter dependent on i) the number of scratches, ii) the depth of the scratches and iii) the density of the scratches. The grade 2 specimens, therefore, probably covered a wider range of damage with the grade 1 and 3 specimens only covering the extreme ends of the damage spectrum.

However, this provided no explanation for the apparently higher mean value for the grade 2 specimens compared to the grade 3 specimens, for example. No firm conclusions can be drawn, but there exists the possibility that damage in the grade 2 specimens, being significantly deeper than the superficial zone (and the fine scratches observed in grade 1 specimens), encroached upon the thickness of cartilage where the chondroitin sulphate and keratan sulphate content increased rapidly with increasing depth. Hence, additional damage to the cartilage, caused during the wear test would release significantly greater amounts of sulphated GAGs compared to normal or finely scratched grade 1 specimens. However, such an increase in the concentration of sulphated GAGs present in the lubricant of grade 3 specimens was not recorded, even though, considering figure 4.30, the total GAG concentration continues to rise with increased depth of cartilage.

The data shown in figure 4.30 relate to intact, undamaged cartilage. Freeman and Meachim (1979) stated that the proteoglycan content in cartilage will progressively decrease with increasing fibrillation of

the cartilage. Although, it could be argued that there is a difference between the classical definition of fibrillation (see section 2.4) and the description of the parallel scratch marks, there is no doubt that both descriptions define cartilage that is damaged. It will be seen later (section 4.6.19) that in the SEM the parallel scratch marks described had similar characteristics to that of a fibrillated articular surface. It is possible, therefore, that decreased levels of proteoglycans would be measured in the more damaged grade 3 specimens.

Freeman and Meachim (1979) also stated that the rate of proteoglycan *synthesis* is below normal in severely fibrillated tissue and either normal or above normal in minimally fibrillated tissue. It is conceivable, therefore, that cells within the grade 2 scratched cartilage were attempting repair and thus synthesizing proteoglycans at a faster rate than normal *in vivo*, hence the increased sulphate concentrations recorded, but that the rate of synthesis *in vivo* in grade 3 specimens was abnormally low, hence the reduced concentration of sulphated GAGs measured in the cartilage debris compared to that of the grade 2 specimens.

Alternatively, the presence of many fine scratches may be more disruptive to the articular surface than a few deep score marks, as in the grade 3 specimens. These are all unsubstantiated postulations and it is clear that

further investigation is required before any satisfactory explanation can be given.

The LVDT traces for each of the scratch grades are shown in figure 4.32a-c. Figure 4.32a is the mean plot of three separate LVDT traces for three grade 1 specimens, figure 4.32b is a single trace of a grade 2 specimen, and figure 4.32c is the mean of four traces for grade 3 specimens. The standard errors for each mean point are also given for grades 1 and 3. All three traces are plotted together with that obtained previously for the grade 0 specimens (section 4.6.8) in figure 4.33. It is clear from this diagram that there was no significant difference in the vertical displacement recorded during the wear test between any of the four grades, assuming, of course, that the single trace obtained for the grade 2 specimen was representative of all grade 2 specimens. This trace corresponded to that obtained for the specimen that had the highest inorganic sulphate content at 8244ppm. It seems likely, therefore, that, as for the results comparing no crystals to crystals in the lubricant, the LVDT showed no significant difference between the overall vertical displacement of the scratch grades.

These results clarify the evidence produced in section 4.6.12 that vertical displacement, as a measure of wear, was not sensitive enough to detect differences in the wear behaviour of cartilage under varying conditions, as

observed by chemical analysis of the concentration of wear debris in the lubricant with ion chromatography.

Two possibilities exist to explain this anomaly between these two measurements of wear, or damage, to the cartilage. First, that the overall thickness of cartilage removed was not significantly different for any case (as indicated by the LVDT data) but that separate deeper grooves, caused by the crystals, allowed the release of more cartilage debris with the potential of greater GAG concentrations having been removed from deeper within the cartilage thickness (as discussed in section 4.6.12 and illustrated in figure 4.30). Alternatively, cartilage debris could have been generated from outside the contact zone, i.e. from cartilage not being directly worn by the counterface but still immersed in the lubricant. Further data concerning this hypothesis will be discussed later in section 4.6.17.

4.6.14 Creep of Normal Articular Cartilage and Cartilage Scratched *In Vivo*

The creep response of normal articular cartilage compared to that of cartilage scratched *in vivo* was investigated with the LVDT. Sokoloff (1966) showed that the indentation/time characteristics of fibrillated articular cartilage were significantly different from those of normal articular cartilage. Kempson (1979) also showed that the creep modulus was linearly dependent on

the total GAG content of the tissue. It was considered, therefore, that an investigation of the creep response of the scratched cartilage compared to normal cartilage may have revealed important information with respect to the nature of the scratched cartilage.

Figure 4.34-d illustrates the creep response over ten minutes for normal and scratched articular cartilage. Figure 4.34a is the mean of ten LVDT traces for normal (grade 0) cartilage, figures 4.34b-d are the means of three traces for grades 1,2 and 3, respectively. The standard errors for each mean point are also plotted.

There was no significant difference between the creep response over ten minutes for any of the scratched cartilage samples compared to that of normal undamaged cartilage. Sokoloff showed (1966) that the difference in indentation behaviour between fibrillated and normal articular cartilage was significant. The results given in figure 4.34, therefore, indicated that the scratched cartilage behaved differently from fibrillated cartilage. It is interesting to note here that by eye the scratched surfaces appeared quite different from a typical fibrillated surface in that the articular surface between the well-defined scratch marks appeared relatively intact compared to the general overall roughness of a fibrillated surface. Indeed, such parallel scratch marks observed *in vivo* in equine fetlock joints are reported to be clinically asymptomatic (Barr, 1992).

On reflection, it was considered probable that the LVDT system used in this experimental setup would not be sensitive enough to detect any change in behaviour of the cartilage owing to any alteration in the GAG concentration in the tissue as described by Kempson (1979).

4.6.15 Comparison of the Creep and Consecutive Wear Response of Normal Articular Cartilage

As discussed briefly in section 4.6.8 it was observed that the vertical displacement of the cartilage due to creep was greater than that of the wear curve measured with the LVDT for the first five minutes of the wear test (see figure 4.17) for the same specimen. This phenomenon was unusual in that during this short time period it would be expected that the wear and creep curves would coincide or that the displacement due to wear, with components of creep and wear, would be greater than the displacement due to creep, not less. This phenomenon was therefore investigated further.

The only difference between the wear and creep situations was that the counterface rotated relative to the cartilage for the wear response whereas everything was static for the creep response. A creep/wear/creep experiment was carried out where the creep response of a specimen of normal equine articular cartilage was recorded for twenty minutes. The counterface was then

rotated, slowly at first but gradually reaching the testing speed of 85mm/s over approximately three minutes, and the cartilage was allowed to wear for twenty minutes after which the rotation of the counterface was gradually stopped (again over approximately three minutes) and a second creep curve for a further twenty minutes was recorded. The vertical displacement of the cartilage was recorded with the LVDT. Three specimens were tested and a typical trace showing several characteristic features is given in figure 4.35. First, note the decrease in the vertical displacement recorded at the start of the wear test, as the plate reached normal testing speed. Second, note the approximately horizontal wear curve during the remainder of the twenty minute wear test, and third, note that the second creep phase appeared to be a direct continuation of the first, after a small initial more rapid response. This last characteristic is illustrated in figure 4.36, where the two creep responses have been superimposed and it can be clearly seen that the creep response was continuous. Indeed, the horizontal wear curve indicated that no wear, but also no displacement due to creep, occurred during these twenty minutes.

The decrease in the vertical displacement of the cartilage specimen at the beginning of the wear phase was difficult to explain. It was postulated that the effect was due to a relaxation of the cartilage from resorption of fluid. This would have been associated

with a fall in the stress level within the cartilage, an increased pressure within the lubricant possibly due to the hydrodynamic effects of the lubricant moving relative to the cartilage, or an increased permeability of the cartilage such as would occur if the thin superficial zone were removed. It was also possible that such relaxation of the cartilage during the beginning of the wear phase was reflected in the initial more rapid response at the beginning of the second creep phase before this became continuous with the first creep response. If reimbibation of the cartilage is considered as the mechanism by which the cartilage relaxes, then it also becomes possible that the horizontal wear curve observed in figure 4.35 could be a result of equilibrium of wear (increasing vertical deformation) and reimbibation (decreasing vertical deformation).

In order to ascertain whether this phenomenon (of a decrease in the vertical displacement recorded at the beginning of the wear phase) was a general characteristic of the apparatus or was specific to the cartilage, the same experiment was carried out on an ultra-high molecular weight polyethylene (UHMWPE) pin machined to give a contact area, and hence a nominal contact stress, similar to that for the cartilage. The UHMWPE pin was then tested under exactly the same conditions as that for the cartilage. The experiment was repeated three times and a typical trace is shown in figure 4.37. In this case there does not appear to be a

clearcut fall in the vertical displacement recorded with the LVDT. Also, there was no horizontal wear period indicating that the UHMWPE was worn during this twenty minute period, apparently unlike the cartilage. Experiments were also carried out to ascertain whether there was an inherent movement within the bearing of the rotating plate. The plate in its container (see figure 4.1 of the apparatus) was loaded by means of a large steel plate placed across the container. A small fall was recorded of approximately 3μm. However, this would have been accounted for during the creep response before the wear was commenced. It seemed probable, therefore, that the characteristics discussed above, and illustrated in figure 4.35, for cartilage were specific to that material and were not a function of the apparatus.

The fall in the measured vertical displacement of the cartilage was, therefore, a function of the material and the dynamic situation of a rotating plate and lubricant. The same phenomenon occurred when the experiment was repeated over a six hour period to correspond with the time taken for a wear test (see figure 4.38). There was still a fall in the displacement measured with the LVDT at the start of the wear period, and the second creep phase after the wear was continuous with the first. However, as would be expected, the wear curve was not horizontal and was presumably a true measure of the wear, without the creep component, as was discussed in

section 4.6.9. Future experiments should consider the development of this technique to obtain a true measure of the vertical displacement due to wear alone.

Linn (1967) and Lipshitz and Etheredge (1984) also observed a phenomenon similar to that described above when they measured the deformation of cartilage during experiments to measure the friction and deformation of the cartilage. By means of a pendulum-type apparatus called an arthrotripsometer, Linn (1967) observed that when a constant load was applied to cartilage first an initial and then a creep deformation occurred which progressed for as long as 24 hours (under a maximum pressure of 27.5kg/cm^2 (27.5kPa)). However, when the joint was oscillated the amount of deformation became constant, arriving at a load-specific value within five to six minutes. Linn (1967) also observed that if the constant load was first applied long enough for the deformation to exceed the load-specific value had the joint been oscillating, that when oscillation was started the deformation rapidly *decreased* to the load-specific value. This took approximately five minutes for a 40lb load (about 18kg).

These observations are in good agreement with the data given in this thesis, in that a decrease in deformation was observed upon relative movement of the cartilage with the opposing surface. However, the relative motion described in this thesis was not oscillatory as described by Linn (1967) and the opposing surface was

stainless steel, not cartilage. Linn (1967) discussed his observations with respect to the mechanical extrusion and resorption of interstitial fluid and concluded that the oscillation reduced the recovery time of the cartilage for the following reasons. First, at some part of each cycle of oscillation approximately half of the portion of the (talus) cartilage surface, which carried the load, was exposed to the immersion of the fluid, and hence it was assumed that fluid would be resorbed into this portion of the cartilage thus allowing its recovery. Second, there was a flow of interstitial fluid through the colloid matrix which was proportional to the difference in hydrostatic and osmotic pressures within the cartilage and across the opposed surfaces of the cartilage. This would supply fluid to those regions of the cartilage which were not exposed directly to free fluid during oscillation. Linn's first explanation of this particular phenomenon cannot be valid for the phenomenon described in this thesis as the loaded area is always in contact with the rotating counterface and so resorption of fluid into a cyclically unloaded portion of cartilage is impossible. Hence the oscillatory nature of Linn's system cannot be an important factor in the explanation of this phenomenon. Linn's second explanation, that a pressure gradient is set up within the cartilage to enable fluid flow, is plausible, however, but Linn does not continue to explain how this difference in pressures would be achieved specifically in the dynamic situation. It could

be imagined that some sort of internal "pump" is created by the differences in the hydrostatic and osmotic pressures as the joint oscillates, (see figure 4.39), but it is again difficult to imagine how such a configuration could be applied to the situation described in this thesis where the cartilage is loaded in one place only against a constantly rotating counterface.

Lipshitz and Etheredge (1984) also observed such a phenomenon when wearing cartilage against formaldehyde-reacted cartilage with interrrupted oscillating sinusoidal motion. When the sinusoidal motion was resumed after interruption the compressed dehydrated regions of the cartilage reswelled. Unfortunately, Lipshitz and Etheredge (1984) gave no explanation of this phenomenon beyond stating that they believed that their data indicated that wear rates of articular cartilage were functions of its swelling rates, fluid exudation rates and states of hydration, among other factors.

In the case described in this thesis, the only differences between the static and dynamic situations, as stated previously, were the presence of a counterface and fluid moving relative to the cartilage. Three explanations have already been suggested: reduced stress concentrations within the cartilage; the existence of an external hydrodynamic pressure; and the removal of the less permeable superficial zone. It was assumed that a

hydrodynamic pressure was set up within the fluid as it approached the gap/contact area between the cartilage and the counterface (see figure 4.40) (Nordin and Frankel, 1989). This hydrodynamic pressure was necessarily greater than that existing in the static situation (which was equal to zero) and the state of equilibrium between the cartilage and the fluid would therefore have been disturbed. It was suuggested that cartilage, being a porous, permeable material, responded to this additional external pressure by reimbibing more interstitial fluid to equilibrate once again the external and internal pressures. However, this assumed that the stress concentrations within the cartilage remained the same for the dynamic situation, where the cartilage was also subject to shear stresses. Stress analysis by Mohr's circle (figure 4.41) indicated that the maximum stress concentration within the cartilage would increase when the material was subjected to shear forces in addition to compressive loading. However, as a relaxation of the cartilage was observed (which was assumed to be a function of reimbibation of fluid) it must also be assumed that the hydrodynamic pressure generated in the fluid was greater than any increased stress concentrations within the cartilage for this situation to exist.

Alternatively, the thin superficial zone (approximately 8μm thick) may have been removed during the first few minutes of the wear test, thus removing the membrane

which is considered by some people to control the rate of fluid flow in and out of the cartilage (Lipshitz *et al*, 1975, Mow & Mansour, 1974). The internal region of the cartilage, being more porous, could therefore reimbibe fluid in a greater quantity than with the superficial layer intact and hence the cartilage could swell, thus reducing the measured vertical displacement. If this were true the phenomenon would only be observed with new, undamaged pieces of cartilage. A cartilage specimen that was retested following a creep/wear/creep test showed only a very slight decrease in the measured vertical displacement at the begining of the second wear phase compared to the first, (see figure 4.42), indicating that the removal of the superficial zone that controlled the rate of fluid flow could be a partial explanation of this phenomenon. However, in the case of the experiments carried out by Linn (1967), in particular, it is not clear whether the cartilage surface would have been damaged to this extent. As the experiments were conducted, primarily, to measure the frictional properties of cartilage it is unlikely that the cartilage was worn to any extent. The removal of the superficial zone, although a possible explanation for the phenomena described in this thesis, probably did not occur in Linn's experiments. It seems most likely, therefore, that the relaxation of the cartilage, upon relative motion of the cartilage with the opposing surface, was due to an increased external hydrodynamic

pressure causing further reimbibation by the cartilage matrix.

4.6.16 Transmission Electron Microscopy of Transverse Sections of Equine Articular Cartilage

Transmission electron microscopy of transverse sections of articular cartilage taken from an equine fetlock joint was used to determine the approximate thickness of the superficial zone. This zone consists mainly of closely packed collagen fibres orientated parallel to the articular surface and very few proteoglycans (and therefore GAGs) and is thought to represent between 5 and 20% of the total thickness of the cartilage (Meachim & Stockwell, 1979 and Nordin & Frankel, 1989). The maximum vertical displacement recorded with the LVDT during the wear tests was about 125μm, which represented approximately 10% of the total cartilage thickness. As the amount of wear was measured by analysis of the concentration of sulphated GAGs from the cartilage debris it was considered necessary to determine the thickness of the superficial zone for equine fetlock articular cartilage in order to ascertain the proportion of cartilage worn away that would contain little GAG.

A layer of cartilage below the surface, approximately 4μm thick (but up to 8μm in places), was clearly identified where the collagen fibres were predominantly orientated parallel to the articular surface (see figure

4.43). The sharp demarcation shown in this figure between the layer containing the parallel collagen fibres and that containing the chondrocyte was not always present. Sometimes this layer was seen to consist of an upper zone (approximately 500nm thick) with finer fibrils and a lower zone (approximately 4μm thick) with thicker fibrils. There was also some evidence of a composite layering effect with fibres apparently orientated at angles to one another (see figure 4.44). An amorphous layer was also often observed on the surface of the articular cartilage (figure 4.45). This was presumably the hyaluronate residue from the synovial fluid (Meachim & Stockwell, 1979).

It was concluded from this information that such a thin superficial zone represented only a small proportion (between about 3 and 10%) of the total thickness of cartilage removed and, therefore, only a small proportion of the debris would not be accounted for by analysis of the sulphated GAGs.

4.6.17 Optical and Scanning Electron Microscopy of Cartilage Surfaces Worn With Crystals Present in the Lubricant

Optical (OM) and scanning electron microscopy (SEM) of the worn cartilage surfaces was carried out in order to ascertain the type of damage caused by the different crystal types. Figure 4.46 illustrates the optical

micrographs obtained of the cartilage surfaces with and without crystals in the lubricant. The oval-shaped flattened contact area can be clearly identified. This was characterised in all cases by parallel scratch marks and raised blisters, which are more clearly illustrated in figure 4.47. Evidence of chondrocytes was also observed in all cases indicating that the superficial zone defined in section 4.6.16 had been worn through.

The Raised Blisters

The raised profile of the blisters was confirmed by eye and, as already stated, occurred in all cases with and without crystals. However, when no crystals, or HA or o-CPPT crystals were present these blisters were much better defined than for the m-CPPD crystals (see figure 4.48). For no crystals and HA crystals the surface of the blister appeared similar to that of the surrounding cartilage with scratch marks continuing across the blister. In contrast to this, the surface of blisters created with o-CPPT crystals present appeared fibrillated or tufted at higher magnifications (see figure 4.48) while those for m-CPPD crystals appeared smoother than the surrounding cartilage (see figure 4.48).

It was suggested that the blisters could have been due to a loosening of the underlying collagen network allowing abnormal PG expansion resulting in increased local swelling of the cartilage (Mow *et al*, 1989).

Freeman & Meachim (1979) also suggested that swelling of the cartilage could only take place if the collagen network had first become abnormally distensible. Swelling of the tissue would therefore seem to imply an abnormality of the fibre network. This hypothesis is partially supported by later work (see section 4.6.18) where blisters were observed to be better defined for cartilage subjected to a higher nominal contact stress (2.7MPa compared to 0.7MPa). Lipshitz *et al* (1980) concluded that swelling ratios of cartilage decreased with increased interaction between the matrix constituents (i.e. increased cross-link density). In the above hypothesis the opposite has been assumed, i.e. that swelling ratios increased with decreased interaction between the matrix constituents.

Parallel Scratch Marks

Parallel scratch marks were visible in all cases (figure 4.49), but they were much better defined for no crystals, HA and o-CPPT than for m-CPPD crystals. Those when no crystals were present in the lubricant were clearly visible and approximately 25μm wide. Smoother marks alternated with ones that appeared rougher with a more open cartilage network. The tracks for the o-CPPT crystals were well-defined with clean edges and were also approximately 25μm wide. However, in this case the cartilage surface appeared generally fibrillated (or tufted), more so on the apparent raised areas but also

within the tracks, but as if the fibrillations had been squashed. The tracks for the HA crystals were also well-defined, but were smoother in apparent profile than those caused by the o-CPPT crystals. They consisted of alternate wide (approximately 40μm) and narrow (approximately 20μm) tracks. The overall surface appeared rough with an open cartilage network similar to that observed on alternate tracks for no crystals. However, in this case the tracks appeared to be superimposed onto this general surface feature. In the case of the m-CPPD crystals, however, tracks were just visible at the lower magnification but this definition was lost at the higher magnification when a uniform but rough open cartilage network was observed similar to that seen for the HA crystals.

First, it is important to remember that only one specimen for each case was examined. Second, all of these micrographs were of the unloaded situation. Any conclusions with respect to potential wear mechanisms were, therefore, drawn with care. These data did provide information, however, that indicated that the type of damage sustained by the cartilage was influenced by crystal size and morphology as the wear tracks produced in all cases were unique.

The o-CPPT crystals produced well-defined tracks, indicating an abrasive wear mechanism (Moore, 1972). There was evidence that the large o-CPPT crystal aggregates were broken up, thus releasing the individual

flat eliptical portions (see figure 4.51). It could be imagined how such a crystal, broad-side on, could create a track as shown in figure 4.49. These large well-defined tracks showed little evidence of any cutting or tearing of the cartilage, suggesting that some sort of ploughing mechanism had taken place. In general though, the cartilage surface had a fibrillated, or tufted, appearance. This may have been caused by the sharp pointed facets of the large crystal star-bursts snagging the cartilage, much as a sharp point snags a textile.

The wear tracks for the HA crystals were again well-defined indicating that some sort of abrasive wear mechanism had taken place (Moore, 1972). The significance of the clearly defined rucking, in this case, will be discussed in more detail later. However, this phenomenon appeared similar to the "abrasion pattern" described by Moore (1972) and Schweitz and Ahman (1986). Moore (1972) stated that such ridges are eventually removed by an abrasive process but are self-perpetuating, so ridges continue to grow from the bulk material. It appears, therefore, that in the presence of HA crystals the cartilage was probably worn by two separate abrasion mechanisms.

In contrast to the other two crystal types there were no clearly defined wear tracks in the case of the m-CPPD crystals. At higher magnifications the surface appeared uniformly rough exposing an open cartilage network. Owing to the lack of wear tracks, indicating that even

those produced with no crystals had been removed by some process, and the relatively small size and aspect ratio of the crystals, it was suggested that some sort of polishing effect could have taken place.

The evidence of a uniform, rough, open cartilage network indicated that wear of the cartilage with m-CPPD and HA crystals, at least, had probably proceded well into the mid-zone where collagen fibres are reported as being relatively coarse (approximately 30 to 80nm) and randomly orientated (Meachim & Stockwell, 1979).

Crystal Debris

Wide, short scratches at an angle to the long parallel scratches were observed on the top of blisters created with HA crystals present (see figure 4.47d). These were possibly an artefact caused by twisting the cartilage specimen when removing it from the wear apparatus, hence possibly trapping crystals, or other debris, between the cartilage and the counterface. However, when apparent crystalline debris adjacent to these scratch marks (figure 4.50) was analysed with energy dispersive analysis (EDA) it was found to contain no calcium. Some of this debris was presumably cartilage fragments, but many particles could not be positively identified.

Debris that was positively identified by EDA as probably being crystalline (in that it contained calcium and phosphorus) is shown in figure 4.51. Both the HA and the

m-CPPD crystals appeared to have aggregated in some form, compared to their morphology prior to the experiments (see figure 4.6). However, the crystal debris shown in figure 4.51 from the o-CPPT experiment was presumably a broken fragment from the original star-bursts. In general, though, very little evidence of crystal debris was observed on the cartilage surfaces.

Rucking of the Cartilage Surface

Rucking of the cartilage surface perpendicular to the direction of motion and wear could be seen to varying degrees in all cases (see figure 4.49 and 4.52). This phenomenon appeared similar to the "abrasion pattern" defined by Moore (1972) where sets of parallel ridges perpendicular to the direction of motion are found on the surface of a rubber or elastomer that has been abraded without a change of direction. Moore (1972) assumed in his theory that this abrasion process was due to tensile failure of the elastomer, with detachment of molecules from the bulk caused by catastrophic tearing behind the sharp asperities in the track, following very high rates of strain. Schweitz and Ahman (1986) make an association between these abrasion patterns and Schallamach waves (parallel regions of contact transversely orientated to the sliding direction and travelling like wave-fronts in the sliding direction observed when rubber slides against a smooth surface

under high friction) stating that "although formed under different conditions of friction and wear, both Schallamach waves and Schallamach patterns are most probably the results of periodic fluctuations between compression and tension, which may occur along the contact surface under certain sliding conditions". Indeed, Courtel *et al* (1975) described such ridges on cartilage surfaces, also comparing them to Schallamach waves observed when measuring the friction of rubber and to the theory of Mow *et al* (1974) who postulated that dynamic interaction of synovial fluid with articular cartilage under certain conditions may create an instability in softened or degenerate articular cartilage resulting in undulations of the surface. Schweitz and Ahman (1986) go on to state that the appearance of these patterns may vary depending on material properties and sliding conditions, resulting in a scale or tongue-like structure, "edge rolls" or a smearing effect of the surface.

It is suggested on the basis of this information and considering figure 4.49 (illustrating both wear tracks and rucking of the cartilage surface with and without crystals present in the lubricant) that for no crystals and m-CPPD crystals there was evidence to suggest that the cartilage surface had suffered from a smearing effect, similar to that observed in illustrations of such an effect on rubber given by Schweitz and Ahman (1986). Figure 4.49h and 4.52 indicate that some sort of

"roll" effect may have taken place when HA crystals were present, whereas the cartilage surface seen in figure 4.49c and d that was worn by o-CPPT crystals has been described as fibrillated, or tufted, which fits none of these descriptions of possible wear mechanisms of rubber. Presumably, this effect was also influenced by the fibrous collagen network present in cartilage.

It was therefore tentatively concluded that cartilage in this *in vitro* situation (i.e. worn against a stainless steel counterface in one direction only, with and without the presence of abrasive particles in the lubricant) behaved similarly to rubber under certain "mild wear" conditions as defined by Schweitz and Ahman (1986) (i.e. a slow rate of material removal) in that an "abrasion pattern", similar to the phenomenon of Schallamach waves (Schweitz and Ahman, 1986), was observed. Evidence of abrasion mechanisms including smearing and "roll effects" were observed as well as wear tracks typical of a more severe abrasive action. Each of these effects were the result of different sizes and morphologies of crystal present in the lubricant. These observations therefore support the hypothesis that different types of crystal influenced the type of wear damage incurred by the cartilage.

Damage of the Cartilage Outside the Contact Area

As already discussed in section 4.6.11 there existed the possibility that the increase in inorganic sulphate

concentration measured in the lubricant by ion chromatography with crystals present was due to damage of the cartilage outside the contact area, i.e. of cartilage not in contact with the counterface but still immersed in the lubricant. The cartilage surrounding the contact area was therefore examined by SEM for any signs of damage. Figure 4.53 shows such areas either side of the contact area and as can be clearly seen damage of the cartilage surface is evident in all cases.

Perhaps surprisingly, even when there were no crystals present in the lubricant there was still damage of the cartilage surface. On one side this appeared as pitting and peeling of the cartilage surface (figure 4.53a) whereas the other side, in comparison, appeared relatively undamaged, although some evidence of peeling was observed with some chondrocytes exposed indicating that the superficial zone (defined in section 4.6.16) had been removed. There was also evidence of chondrocytes just below the cartilage surface (figure 4.53b) indicating a thinning of the superficial zone.

Both sides of cartilage worn in the presence of o-CPPT crystals were damaged (figure 4.53c & d). On one side the collagen network was just visible, indicating that again the superficial zone had been removed, and the majority of the damage reflected some sort of peeling or smearing mechanism which appeared aligned with the direction of motion. Some surface cracks were also apparent and chondrocytes were exposed. On the opposing

side the surface appeared relatively intact but scoured with relatively large, deep cracks also approximately aligned with the direction of motion.

In the case of the m-CPPD crystals the damage to both sides was similar, again consisting of what appeared to be a long, linear peeling effect (resembling the effect of peeling the skin from a mushroom) aligned with the direction of motion. This was a little more pronounced on one side than the other (figure 4.53f).

One side of the specimen worn in the presence of HA crystals (figure 4.53g) appeared very similar to the damage produced when no crystals were present in the lubricant (figure 4.53a) consisting of pitting and peeling effects. The opposing side was relatively undamaged.

The mechanism by which these areas of cartilage became damaged is unclear. It was not solely due to the abrasive nature of crystals in the lubricant as damage was also observed on cartilage that was immersed in a lubricant with no crystals present. Several other possibilities were considered. It seemed unlikely that such damage could have been caused purely by the motion of the fluid. The presence of cartilage wear fragments in the lubricant could possibly have initiated some form of adhesive wear mechanism. This argument obviously would not hold in the *in vivo* situation as the articular surfaces would degenerate rapidly as a result of

cartilage fragments present in the synovial fluid of normal synovial joints (Evans *et al*, 1981). However, *in vivo* the articular surfaces are covered and protected by a boundary lubricant, hyaluronic acid (the high molecular weight constituent of synovial fluid), whereas *in vitro*, in this experiment, such deposits were removed from the cartilage surface prior to the wear test. The relatively clean cartilage surface would therefore be exposed to the lubricant and any loose cartilage debris.

Kempson (1979) reported that the tensile fracture strength of cartilage taken from the superficial zone was strongly dependent on the collagen content and on the extent to which the collagen fibres were orientated parallel to the direction of tension. Freeman and Meachim (1979) and Kempson (1979) reported that cartilage adjacent to areas of fibrillation could show disruption of the collagen network at the ultrastructural level and a reduction in the tensile strength and stiffness of the tissue. However, none of these changes was invariably present and such cartilage could appear to be perfectly normal. It could be imagined that the removal of part of the cartilage surface (see figure 4.54) could severely disturb the integrity of the cartilage structure as a whole. Considering the analogy of a balloon inflated inside a string bag (Dieppe & Calvert, 1983), if part of the string bag is removed, then the rigidity of the structure is lost. Similary, if part of the restraining

collagen network is removed (figure 4.54) it could be imagined how a combination of the compressive loading and an increased internal osmotic swelling pressure (owing to the exposed cartilage matrix absorbing more fluid) could cause the cartilage to distort and swell in excess of normal. This excessive internal pressure could not be constrained as before, owing to the loss of the integrity of part of the collagen network. Hence excessive tensile forces would be imposed on those parts of the network remaining (i.e. in cartilage surrounding the contact area). It can be imagined then how the cartilage surface could begin to disintegrate.

It was also concluded from evidence provided in figure 4.53 that the crystal type had again, influenced the type of damage observed in these regions.

Summary

A complex mechanism of wear was observed to occur both within the contact region and outside. The crystal type appeared to influence the type of damage incurred by the cartilage in both cases. No firm conclusions could be drawn with respect to the type of wear mechanism involved, other than it was obviously abrasive in nature, evidenced by well-defined wear tracks in the cartilage. However, other less severe wear mechanisms, such as "abrasion patterns", smearing and "roll effects" were also observed within the contact area. These reflected the elastomeric wear behaviour of articular

cartilage. Damage incurred by the cartilage outside the contact region, with and without the presence of crystals in the lubricant, was more difficult to explain. However, it was suggested that damage to the cartilage surface in these regions could have been due to the loss of integrity of the cartilage structure as a whole, resulting in excessive internal swelling and hydrostatic pressures within the bulk material and thus abnormally high tensile forces at the surface.

4.6.18 Optical and Scanning Electron Microscopy of Cartilage Worn Under High and Low Nominal Contact Stresses

In order to investigate any differences between the damage sustained by the cartilage at relatively high and low nominal contact stresses (2.7 and 0.7MPa, respectively) optical and scanning electron microscopy of one specimen for each case were carried out. These are shown in figures 4.55 and 4.56. The characteristic features observed were similar to those described in section 4.6.17. However, both the scratch marks and the raised blisters were more defined for the high-stress specimen. Apparent smearing of the cartilage surface was also more evident for the high stress specimen (figure 4.56). Damage of the cartilage surrounding the contact area also occurred. That for the low-stress case appeared less severe, in general, than that for the high-stress case (figure 4.57). The cartilage worn under

a low contact stress suffered from an apparent peeling of a surface layer on one side so revealing the underlying collagen network (figure 4.57a), but apparent pitting of the surface on the other side of the contact area (figure 4.57b). The cartilage under the higher stress also showed signs of peeling on one side but associated with fine cracks predominantly aligned in the direction of motion (figure 4.57c). The profiles of chondrocytes were also surrounded by fine cracks in the cartilage surface. One specific area of this side, adjacent to the contact area exhibited severe cracking of the surface (similar to that observed for o-CPPT crystals) also predominantly aligned with the direction of motion (figure 4.57e). The opposing side also exhibited fine surface cracks (figure 4.57d).

These observations confirmed the fact that cartilage surrounding the contact area could be damaged even with no crystals present in the lubricant. The amount of damage also appeared to be more severe in the case of the higher nominal contact stress. In this case, the presence of surface cracks, fine and deep, supported the hypothesis proposed above that with the integrity of the structure as a whole disrupted, the remaining structure/collagen network would not be able to sustain excessive compressive loads and internal swelling pressures. In the higher stress case damage to the cartilage surrounding the contact area was more severe possibly indicating that the remaining cartilage

structure could not sustain the excessive tensile forces created at the surface and hence splitting and cracking of that surface occurred. The cartilage surface within the contact area of the higher stress also exhibited more well-defined features associated with cartilage damage, such as wear tracks, blisters and smearing/rucking of the cartilage surface. It was concluded, therefore, that the degree and type of damage incurred by the cartilage was dependent on the nominal contact stress. This supported results presented in section 4.6.7 that indicated that the amount of damage of the cartilage (measured by quantitative analysis of the cartilage debris in the lubricant) was directly related to the nominal contact stress applied.

4.6.19 Optical and Scanning Electron Microscopy of Cartilage Scratched *In Vivo*

In order to examine the surfaces of cartilage scratched *in vivo* in greater detail, both OM and SEM were carried out on one specimen for each grade. The optical micrographs shown in figure 4.58 provide a view of the parallel scratch marks similar to that observed by eye. The scratch mark grades were defined as in section 4.4.1: Grade 0 specimens were normal, with no scratch marks visible, grade 1 specimens had very fine scratch marks that were only just visible, grade 2 specimens had fine scratch marks that were easily visible and grade 3 specimens had gross scratch marks. In the SEM the

surfaces looked very different from those observed with OM (figure 4.59) where previously clear parallel scratch marks took on a more typically fibrillated appearance with the fibril direction predominantly perpendicular to the length of the scratch mark. This was thought to reflect the direction of the orientation of the collagen fibre network. Freeman & Meachim (1979) suggested that such parallel track marks could be a result of a three-body abrasion mechanism, possibly caused by crystal-containing cartilage. Our observations indicated that, by eye, no crystals were present in the majority of these specimens. However, the particulate content of the synovial fluids was not investigated.

The obvious preservation of the normal articular surface for the grade 0 specimen provided evidence that the preparation technique used (Cameron *et al*, 1976) did not produce any artefacts. It was therefore concluded with some confidence that features observed on the worn cartilage surfaces *in vitro* and *in vivo* were not artefacts caused by the preparation procedure.

On one area of the grade 2 specimen an apparent peeling of the surface was observed (figure 4.60). The exposed collagen network can be clearly seen. This type of damage was reminiscent of some of the peeling observed on cartilage surrounding the contact area in the *in vitro* wear tests. Indeed other features observed on the surface of this same specimen (figure 4.61) looked remarkably similar to the effect described as "pitting"

in sections 4.6.17 and 4.6.18. There was therefore some evidence to suggest that damage similar to that produced *in vitro* in the wear tests was also produced *in vivo*.

The cause of the damage produced *in vitro* was discussed in terms of the disruption of the collagen network (section 4.6.17s). This hypothesis is supported by Mow *et al* (1989) who provided evidence to support the belief that disruption of the collagen network is a key factor in the initial events leading to the development of osteoarthritis. It is presumed that this would include biochemical as well as mechanical disruption. Freeman & Meachim (1979) described mild superficial fibrillation where there was horizontal splitting at the cartilage surface with the formation of thin strands of tissue, broader-based tufts and small pits. This description of fibrillated cartilage *in vivo* could also be used to describe the cartilage surrounding the loaded contact region that was damaged *in vitro*.

4.7 CONCLUSIONS

A successful technique has been established to measure the wear of articular cartilage by analysing the concentration of cartilage debris in the lubricant. A standard pin-on-disc apparatus was adapted to wear plugs of cartilage attached to subchondral bone against a stainless steel counterface. Ion chromatography was then used to analyse the inorganic sulphate concentration

(derived from the proteoglycans) in the wear lubricants thus providing a measure of the amount of cartilage debris present. Owing to the variable concentration of glycosaminoglycans (GAGs) with depth of cartilage it was difficult to quantify accurately the amount of cartilage removed. It is suggested that any further work analyse the hydroxyproline content (derived from the collagen) which is reported to be constant throughout the depth of the cartilage (Lipshitz & Glimcher, 1979).

Initially, spectrophotometric analysis of the sulphated glycosaminoglycans (GAGs) with dimethylmethylene blue was used to analyse the amount of cartilage debris in the wear lubricant. However, the sulphated GAG concentration recorded was compromised by the presence of crystals. It was concluded that the GAGs were probably binding to the crystals thus inhibiting their detection in the spectrophotometric assay. It was suggested that the protein attached to the GAGs was strongly implicated in such an interaction.

All experiments were conducted under conditions that were as well controlled as possible. Cartilage specimens were taken from the lateral side of equine fetlock joints from the front legs only. Each specimen was graded by eye and only those which were normal were used (apart from those in experiments investigating the wear response of cartilage scratched *in vivo*). A constant speed of 85mm/s and a standard load of 6kg was used for all experiments apart from those investigating the

response of cartilage to varying loads. Unfortunately, though, owing to the variable radius of curvature of the fetlock joints obtained the contact area, and hence the nominal contact stress could not be controlled. Cartilage, as a biological material, is variable in nature from joint to joint and from animal to animal and as such any study of this material will be associated with significant errors. The following conclusions were drawn, however, from investigations completed of the wear response of articular cartilage to varying loads, the presence of crystals in the lubricant and of cartilage scratched *in vivo*.

Experiments investigating the wear response of normal articular cartilage for a range of loads and nominal contact stresses were carried out. It was concluded from these experiments that there was an increasing trend in the relationship between the concentration of cartilage debris measured in the lubricant and the load, and also in that between the concentration of wear debris and the nominal contact stress, i.e. the wear behaviour of articular cartilage was not independent of the apparent contact area. On further analysis of the data, it was also concluded that there was a significant change in the wear behaviour recorded between the loads of 4 and 6kg, and the nominal contact stresses of 1.5 and 1.75MPa. It was suggested that this could indicate the presence of a wear resistant layer which at a nominal contact stress below 1.5MPa remained relatively intact

(under the test conditions described here), but that at higher stresses (greater than 1.75MPa) this resistant layer was broken down, thus increasing the susceptibility of the cartilage to wear. Control experiments indicated that no GAGs leached from intact cartilage when under comparable static loads (ranging from 2 to 10kg).

Investigations of the wear response of cartilage with and without crystals in the lubricant led to the conclusion that the presence of crystals significantly increased the wear of cartilage, as twice the inorganic sulphate concentration was measured in these lubricants as for those not containing crystals. However, owing to the increased concentration of GAGs throughout the depth of the cartilage (Meachim & Stockwell, 1979) it cannot be concluded that twice as much cartilage was removed.

Indeed, results obtained for the vertical displacement of the cartilage during the wear tests (measured with a linear variable differential transducer, LVDT), together with data obtained for the contact areas before and after the wear tests, indicated that the overall volume of material removed was the same for all cases (with and without crystals, and for undamaged and damaged, or scratched, cartilage). It was concluded from these data that the LVDT was not sufficiently sensitive in this experimental setup to detect such differences in the wear response of articular cartilage as observed with ion chromatography. It was postulated that the increased

concentration of cartilage debris measured in the lubricant by ion chromatography could have been derived from either (i) separate, deeper grooves cut in the worn cartilage surface by the crystals which would not be detected by the overall measure of vertical displacement, or (ii) debris from cartilage outside the contact area but still immersed in the lubricant.

Analysis of the inorganic sulphate concentration in the wear lubricants from normal and graded scratched specimens showed no significant difference at the 5% level for all cases. However, it was noted that there was an unusually high mean and large standard error associated with the results for grade 2 specimens, (i.e. with parallel scratch marks easily visible by eye) compared to those measured for grade 3 specimens for which a higher measure of wear might have been expected. It was argued that both the large standard error and the high mean for the grade 2 specimens were potentially important, reflecting several factors, including: (i) the coarse nature of the grading system, (ii) an increased GAG concentration from debris worn from deeper layers of cartilage and (iii) a reduced proteoglycan concentration in fibrillated or damaged cartilage compared to that of undamaged cartilage. The creep response over a ten minute period (measured with the LVDT) showed no significant difference for the scratched specimens compared to normal, undamaged cartilage.

During a study of the creep and wear response of the cartilage with the LVDT it was observed that the creep response was greater than the wear response (which was assumed to be a function of cartilage removal and creep, and would therefore be expected to be the larger). Further investigations of this phenomenon failed to clarify the cause of this anomaly, but it was suggested that the generation of a hydrodynamic pressure in the fluid as it approached the contact area between the cartilage and the counterface could have disturbed the equilibrium of fluid flow in and out of the cartilage so that more fluid could be imbibed by the cartilage causing it to swell.

Transmission electron microscopy of transverse sections of equine fetlock articular cartilage showed the presence of a very thin superficial layer (approximately 8μm) where the collagen fibres ran parallel to the articular surface. This represented only a small proportion of the total thickness of the cartilage (approximately 1mm).

Optical and scanning electron microcscopy of worn cartilage surfaces (*in vitro*) showed that the superficial zone had been worn through by the evidence of chondrocytes at the worn surface. Parallel scratch marks and raised blisters were characteristic features of all the worn surfaces. The cause of the blisters was not clarified but it was suggested that they could have been produced by a mechanical breakdown of the

underlying collagen network caused by excessive shear forces at the cartilage/steel interface, with associated disruption of the proteoglycan-collagen interaction. As a consequence some proteoglycan moieties could have been released enabling them to absorb more fluid, thus causing local swelling. This hypothesis was supported by evidence that showed that blisters created on cartilage surfaces subjected to higher nominal contact stresses (2.7MPa) were better defined than those for lower nominal contact stresses (0.7MPa). The well-defined parallel scratch marks (observed for all cases, with and without crystals present in the lubricant) were indicative of a severe abrasive wear mechanism. Although no firm conclusions were drawn with respect to the specific wear mechanisms involved for the different crystal types it was concluded that the type of damage incurred by the cartilage was influenced by crystal size and morphology as the parallel wear tracks produced in all cases were unique.

Rucking of the cartilage surface, observed to varying degrees in all cases, was compared to abrasion patterns observed on the surface of rubber when abraded in one direction only. It was concluded that in this experimental setup cartilage behaved similarly to rubber under conditions of "mild wear" (as defined by Schweitz and Ahman, 1986). The different types of crystal produced unique mild abrasive effects supporting the

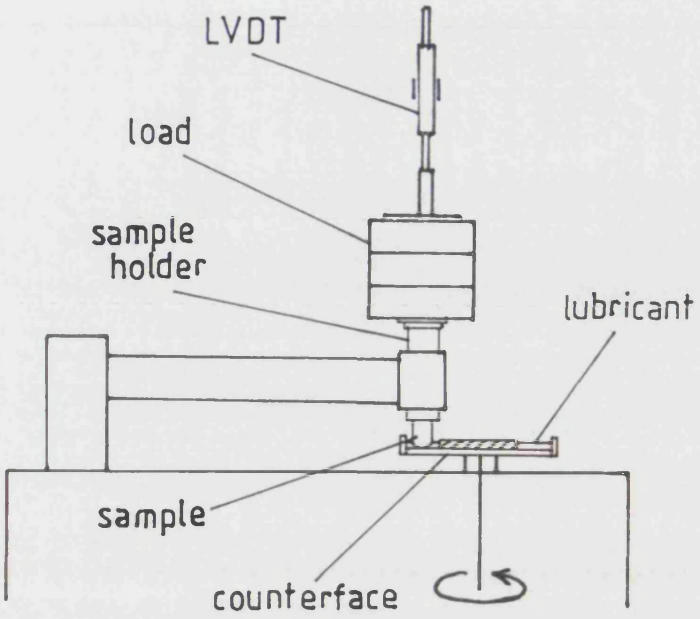
hypothesis that the type of wear damage incurred by the cartilage was dependent on the crystal type.

Investigations of the cartilage surrounding the contact area showed that, although not in direct contact with the counterface, this area of cartilage was also damaged. It was suggested from these data that the cartilage surface could become damaged indirectly as a consequence of the integrity of the cartilage structure as a whole being disturbed, resulting in excessive internal swelling and hydrostatic pressures within the remaining cartilage thus creating abnormally high tensile forces at the remaining cartilage surface.

Scanning electron microscopy of the surfaces of cartilage scratched *in vivo* indicated that clear parallel scratch marks observed by eye consisted of a finely fibrillated surface with loose fibrils perpendicular to the length of the scratch mark. There was also evidence to suggest that damage similar to that produced *in vitro* in this experimental setup was also produced *in vivo*.

These investigations have shown that crystals can cause excessive damage to cartilage and that the type of damage is influenced by the crystal type. They have also shown that damage to cartilage, similar to that produced *in vivo*, can be caused *in vitro*. Perhaps, most importantly, these experiments indicated that such damage of the cartilage surface could occur as a direct

result of mechanical influences only without any associated biochemical response.



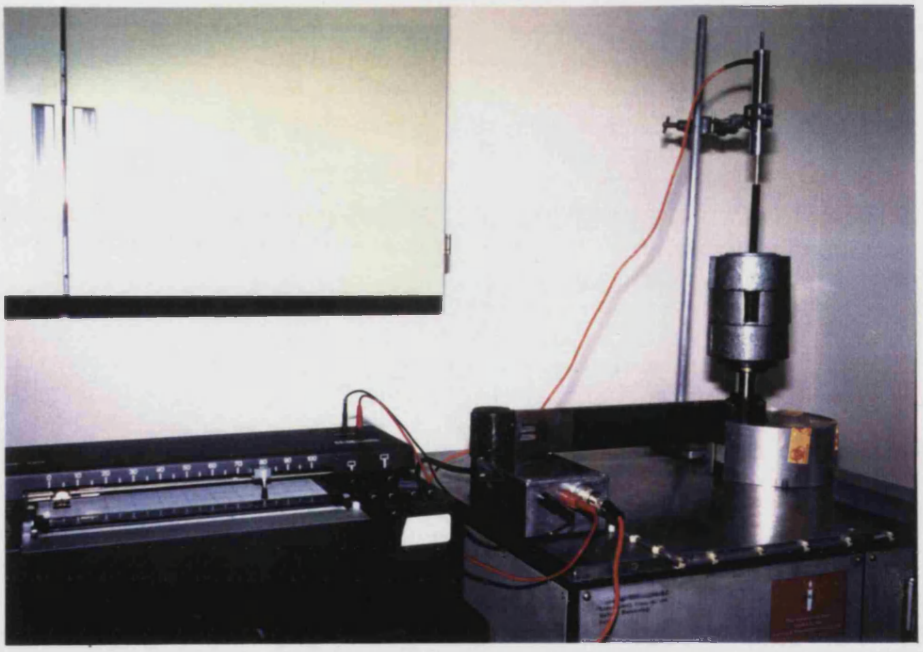


Fig. 4.1 The pin-on-disc wear apparatus.

Fig.4.2 The dissection of an equine fetlock joint.

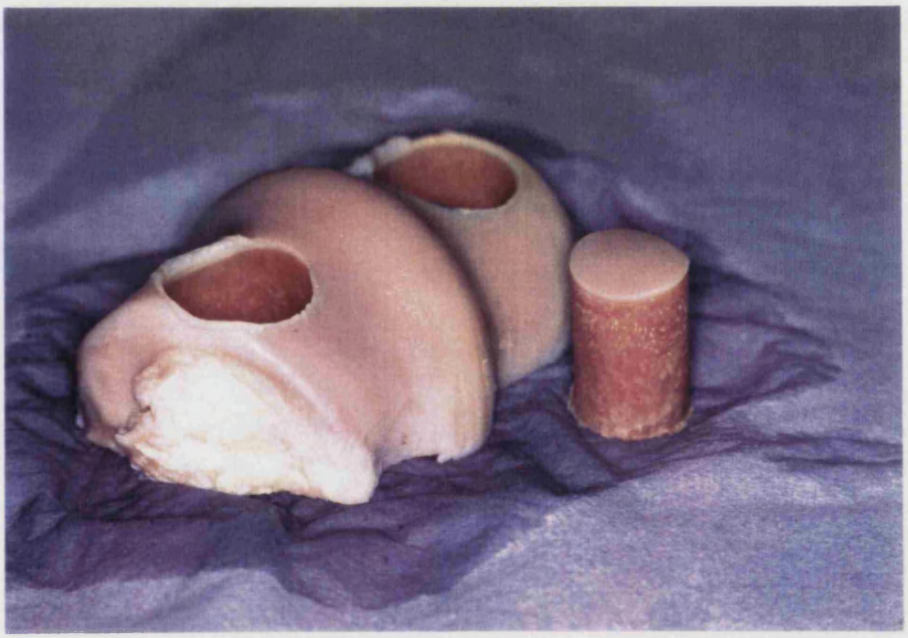


Fig.4.3 A typical cylindrical plug of articular cartilage attached to subchondral bone cut from an equine fetlock joint.

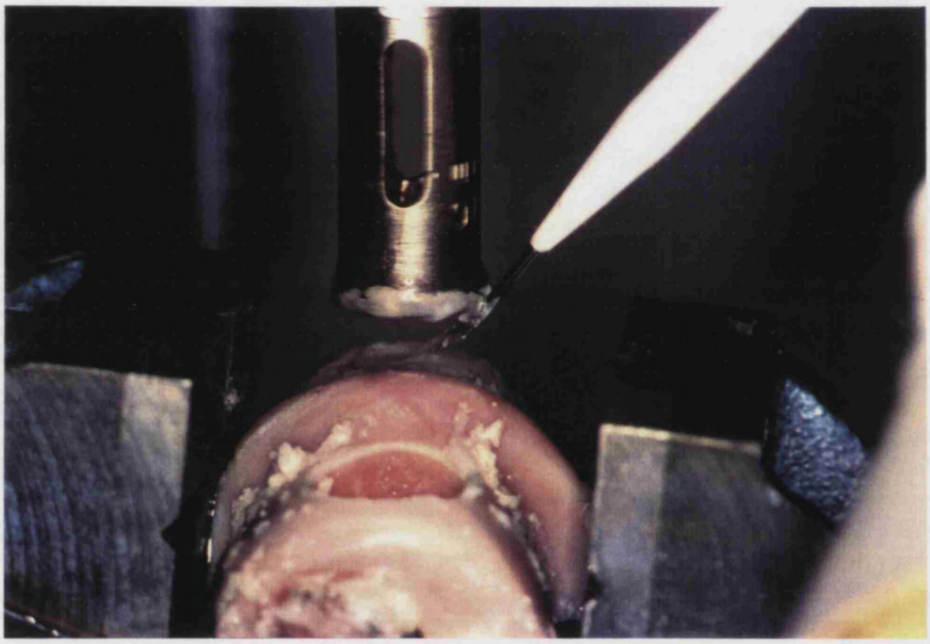


Fig. 4.4 The drilling procedure with distilled water as a lubricant and a coolant.

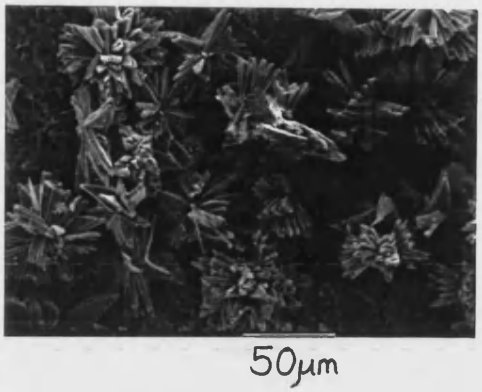


Fig.4.5 SEM of the o-CPPT crystals ("star-bursts") used in the wear experiments.

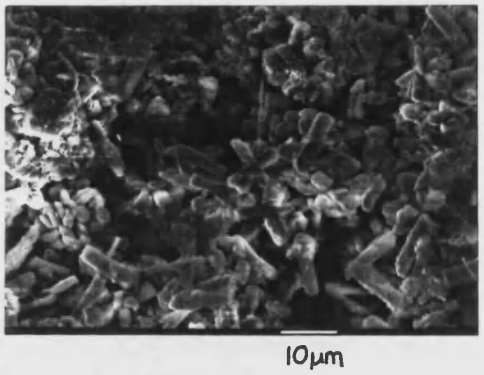


Fig. 4.6 SEM of the m-CPPD crystals used in the wear experiments.

Fig.4.7a TEM of individual HA crystals used in the wear experiments.

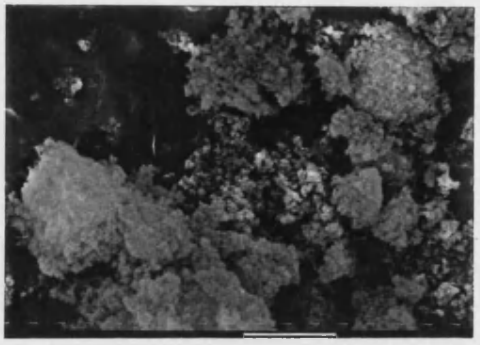


Fig. 4.7b SEM of HA aggregates used in the wear experiments.

Fig. 4.8 SEM of DCPD crystals used in the pilot wear experiments.

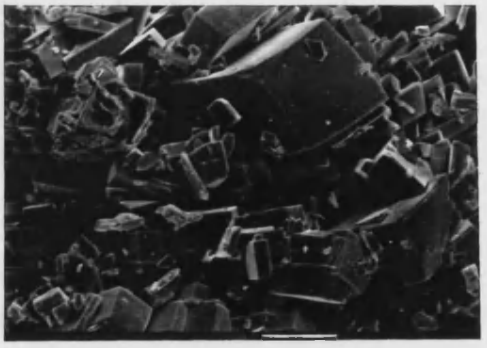


Fig. 4.9 SEM of the CPPD crystals used in the pilot wear experiments.

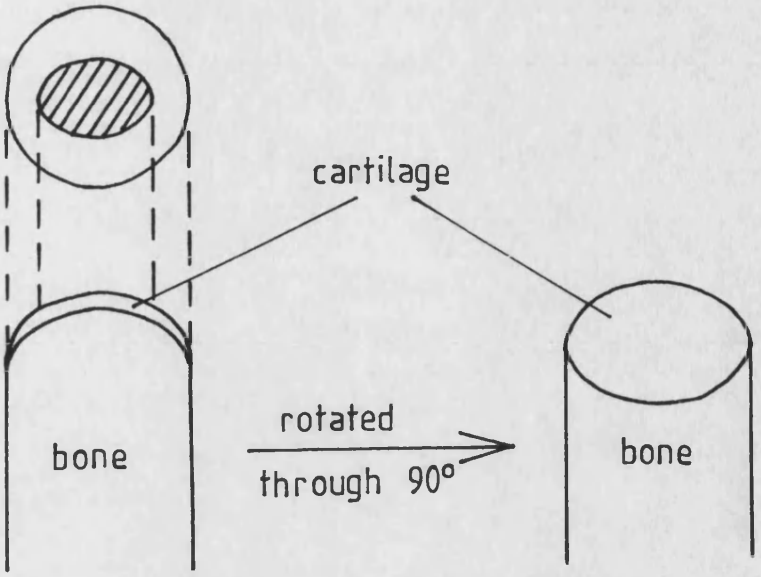


Fig 4.10 Orientation of the curved cartilage surface in the specimen holder.

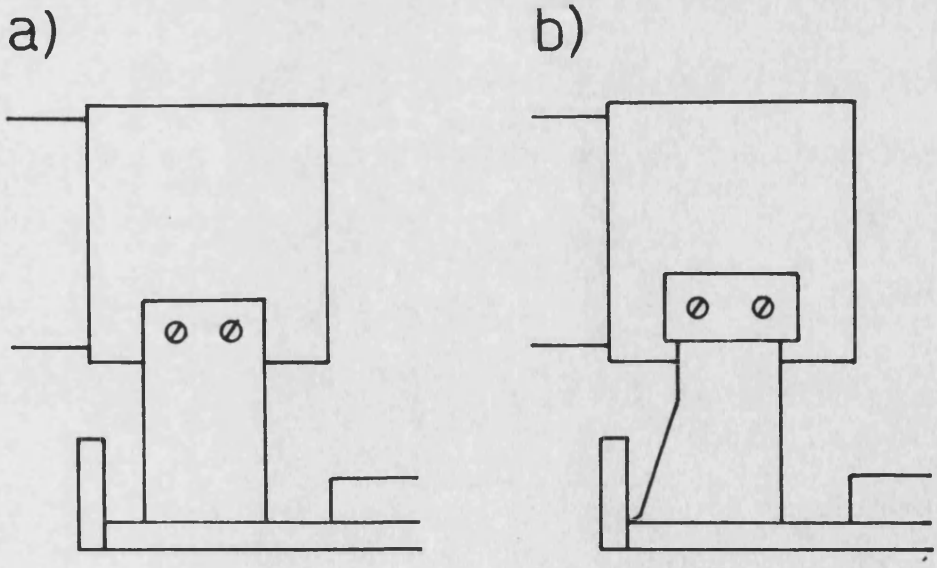


Fig. 4.11 A schematic diagram of a) the plastic paddle, and b) the flexible silicone rubber gate attachments to the counter-balance arm.

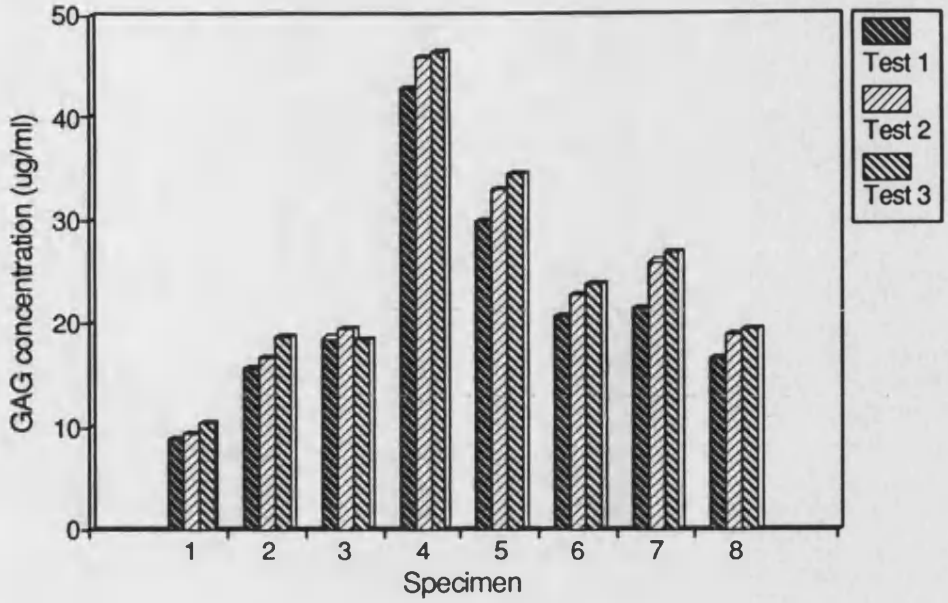


Fig. 4.12 Consecutive measurements of the GAG concentration by spectrophotometric analysis.

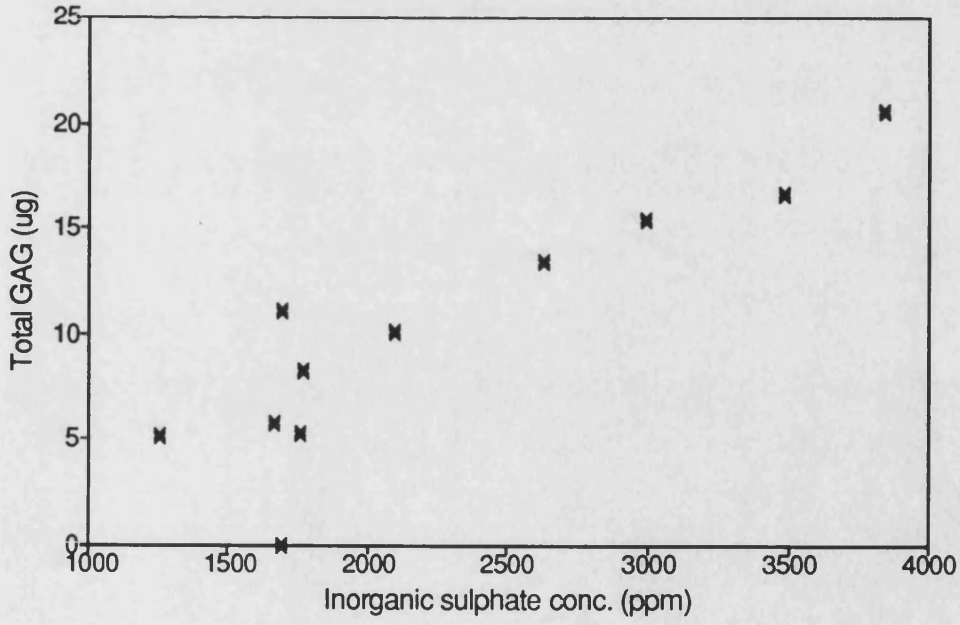


Fig. 4.13 The correlation between spectrophotometric analysis and ion chromatography.

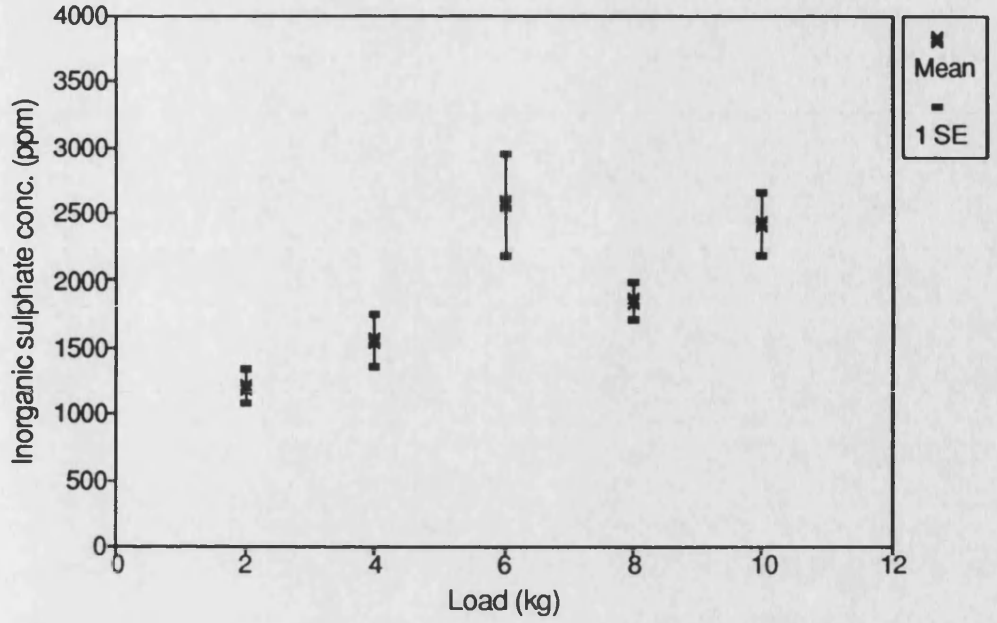


Fig. 4.14a The dependence of wear (inorganic sulphate concentration measured in the lubricant) on load.

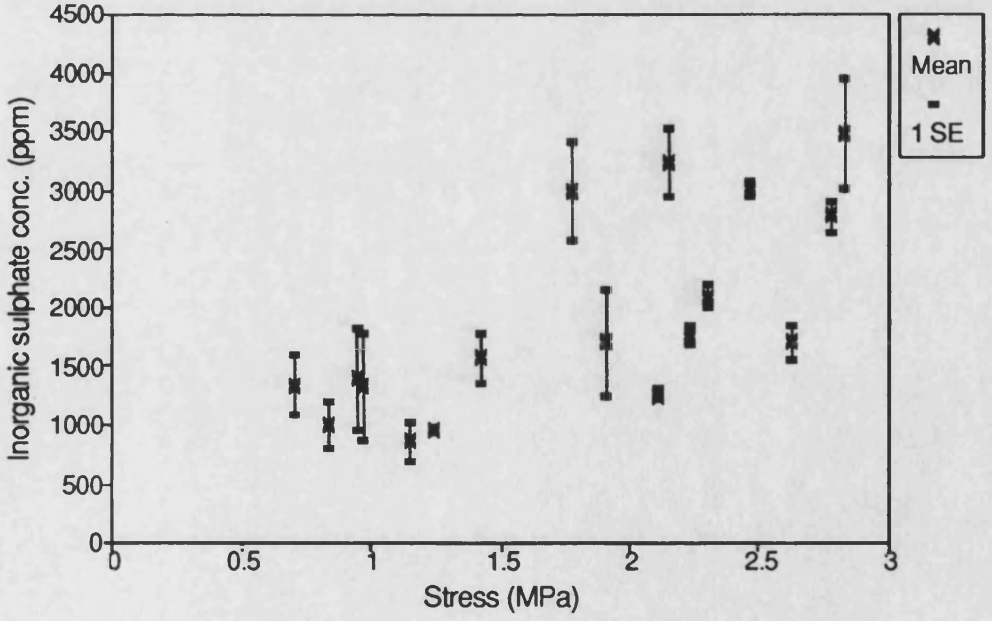


Fig. 4.14b The dependence of wear on the nominal contact stress.

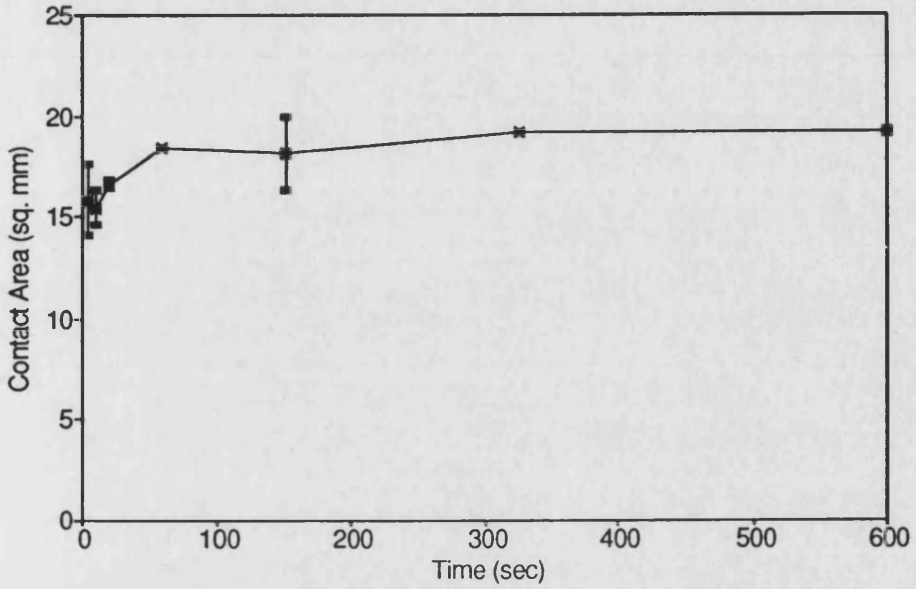


Fig. 4.15 The dependence of contact area on the time during which the load was applied.

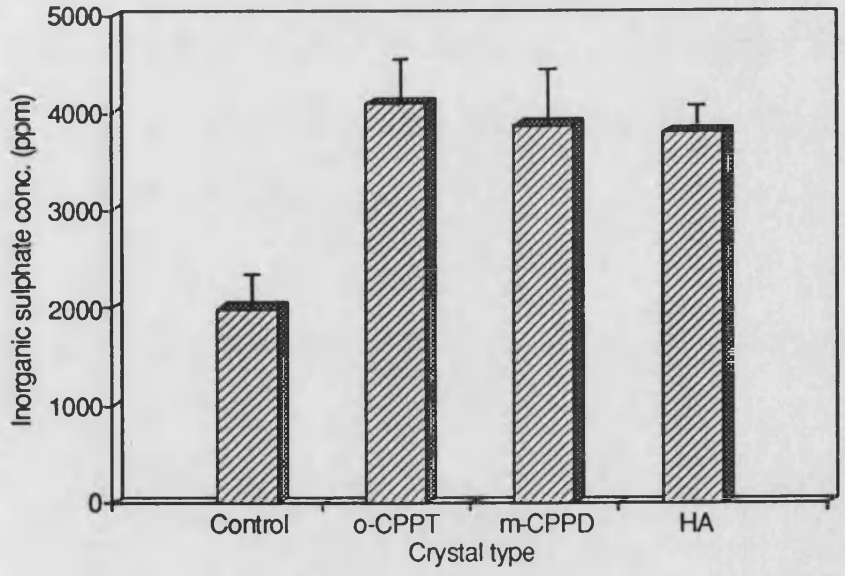


Fig. 4.16 The wear of articular cartilage with and without crystals present in the lubricant.

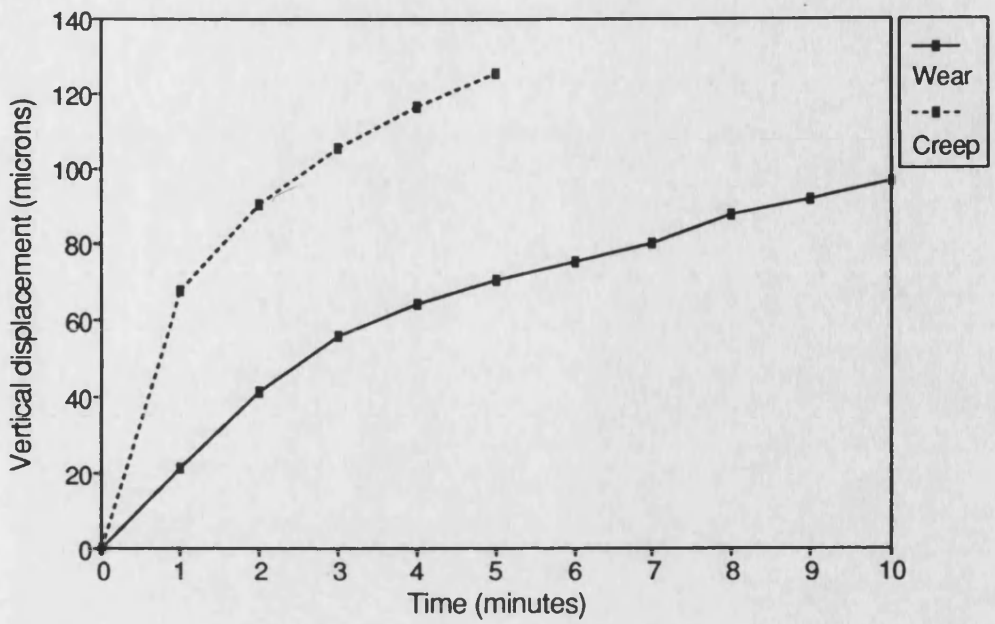


Fig. 4.17 The vertical displacement of articular cartilage due to creep and wear.

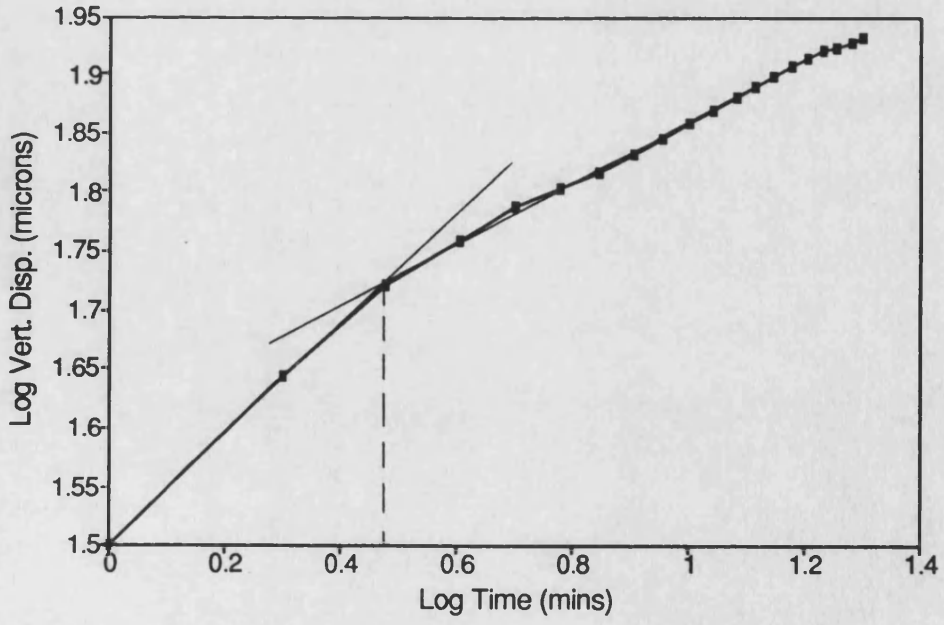


Fig. 4.18 A log/log plot of the vertical displacement during a wear test against time.

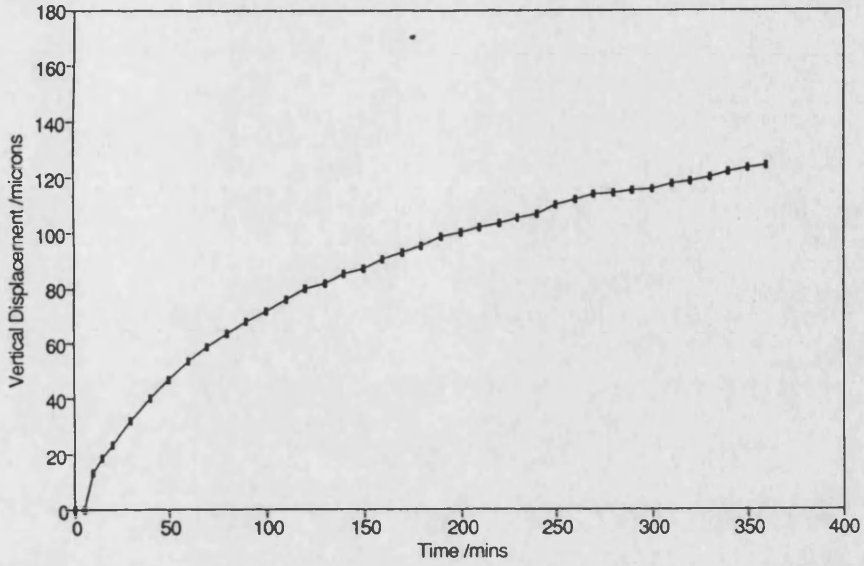


Fig. 4.19 The vertical displacement of a typical specimen of articular cartilage after the subtraction of a nominal five minute creep component.

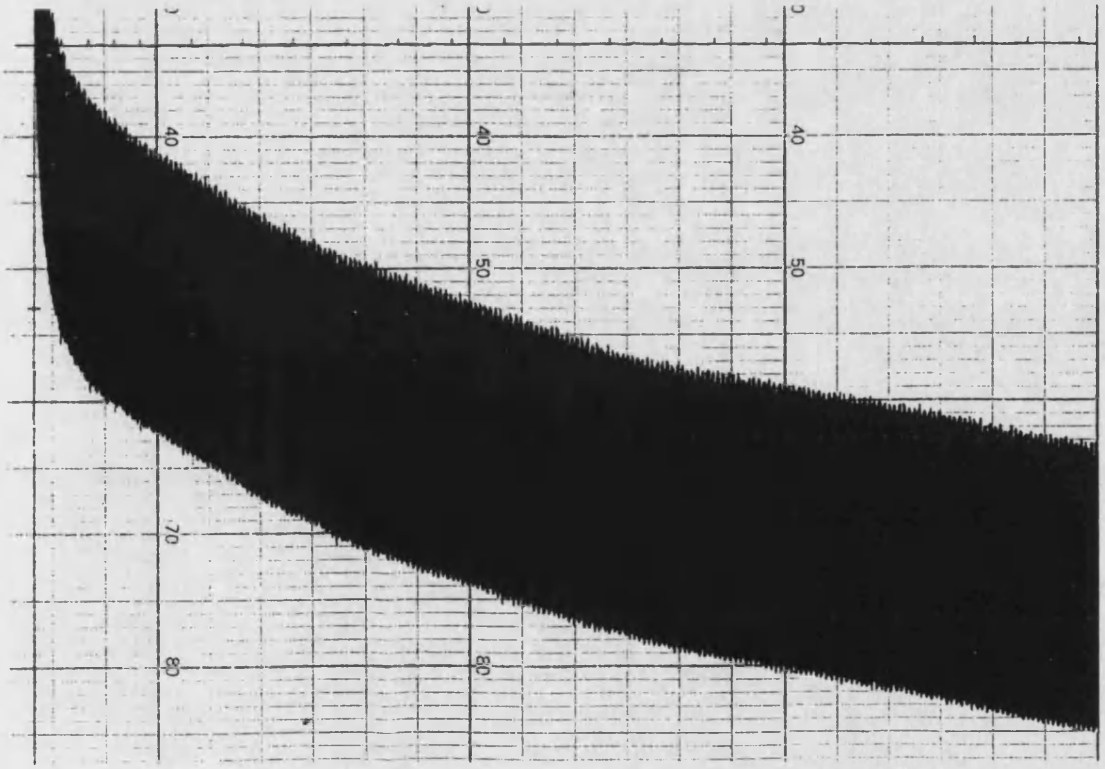


Fig.4.20 A typical chart recorder trace obtained from the LVDT.

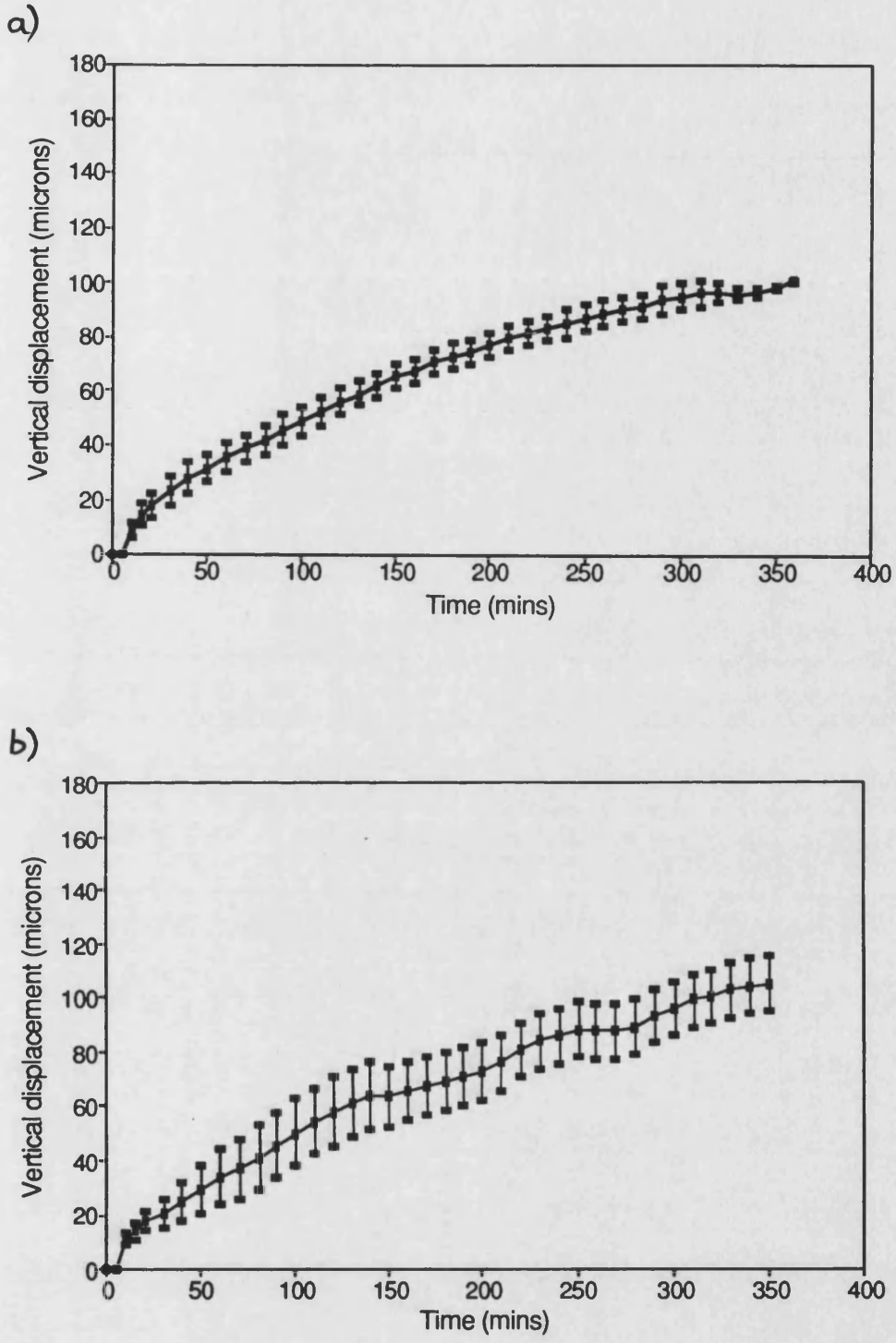


Fig. 4.21 The mean vertical displacement of articular cartilage for a) no crystals and b) 0-CPPT crystals.

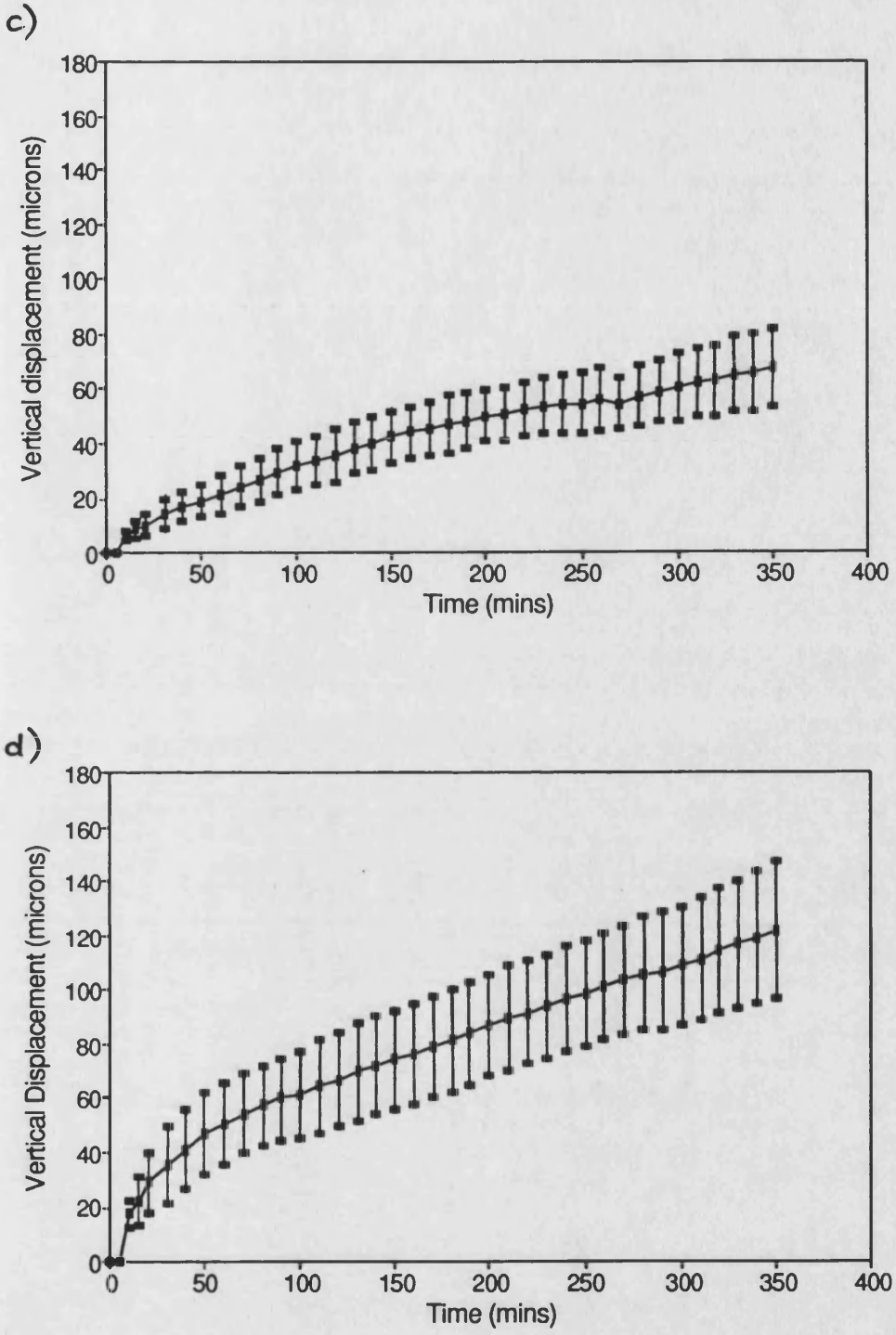


Fig. 4.21 The mean vertical displacement of articular cartilage for c) m-CPPD and d) HA crystals.

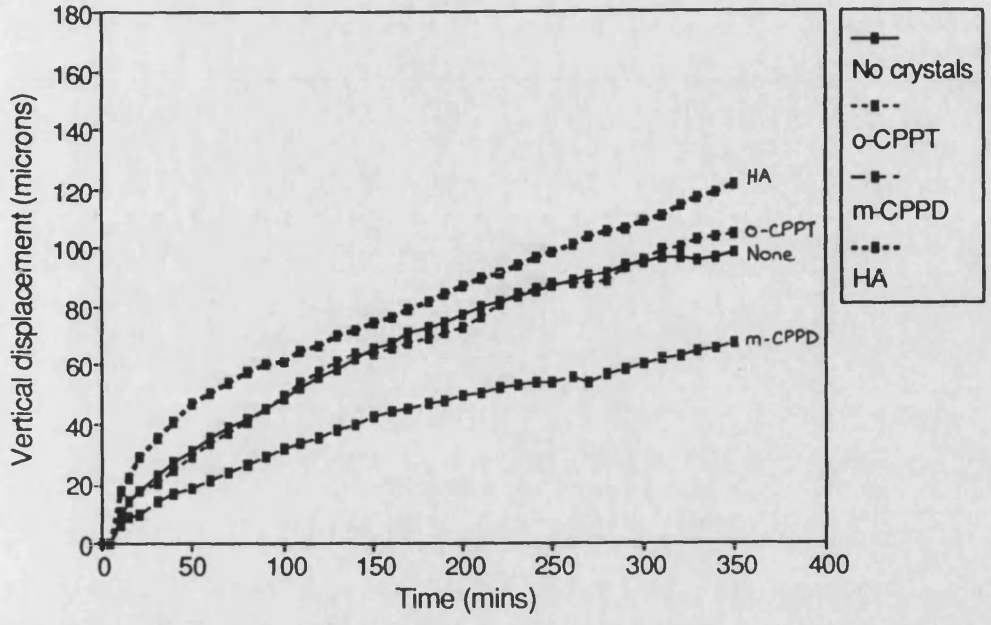


Fig. 4.22 The mean vertical displacement af articular cartilage with and without crystals present in the lubricant.

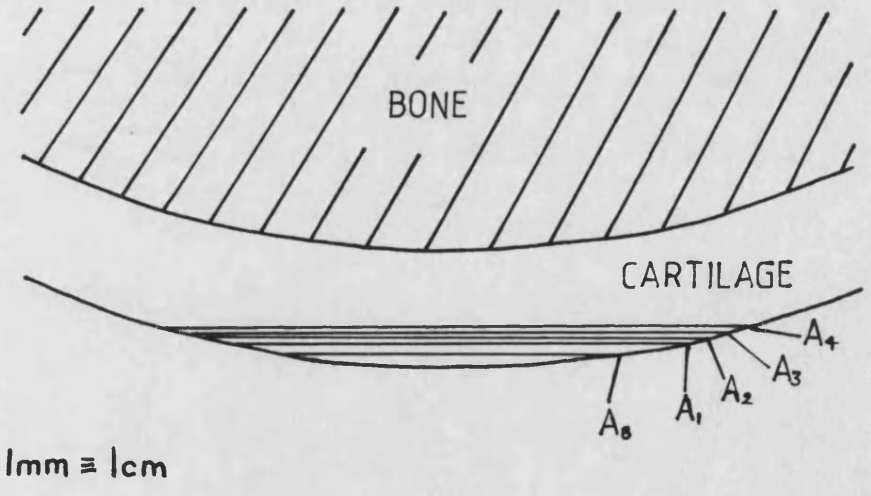


Fig. 4.23 A scale drawing of the relative thicknesses of cartilage removed during hourly periods of a wear test.

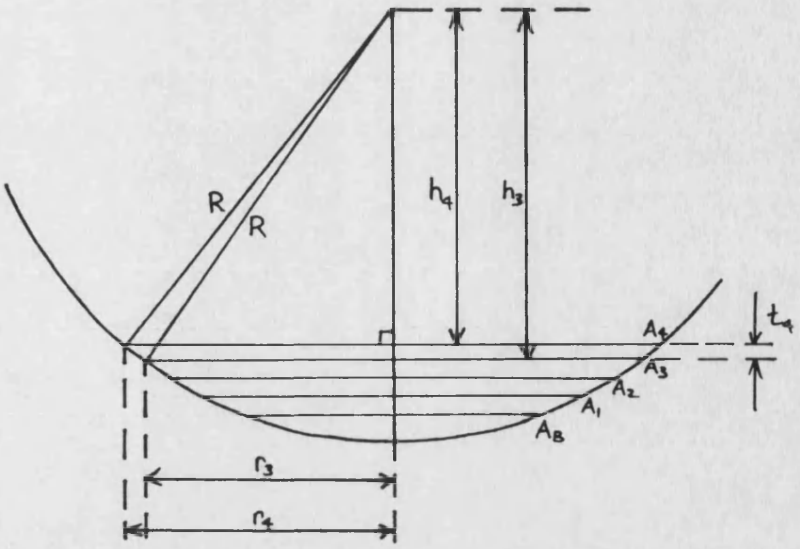


Fig. 4.24a The geometrical construction used to calculate the theoretical thickness of cartilage removed.

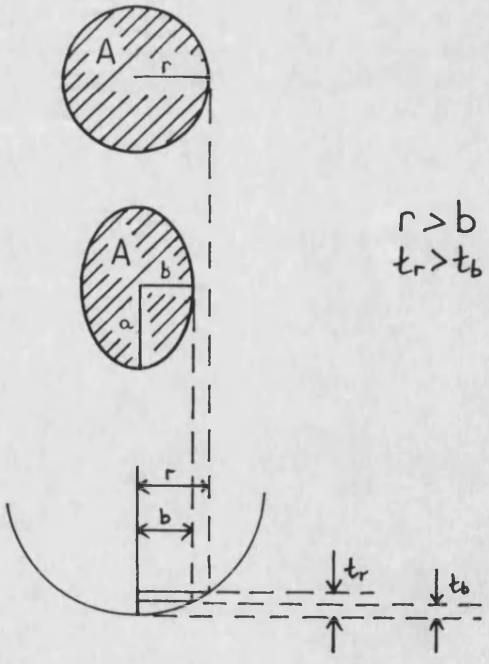


Fig.4.24b A schematic diagram illustrating the error associated with the assumption of a circular contact area.

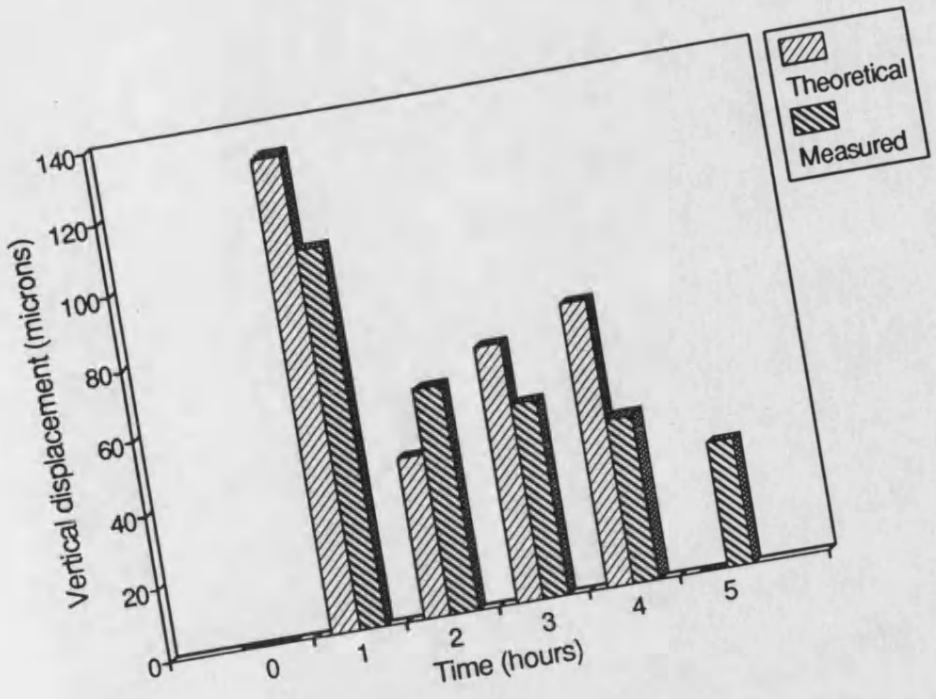


Fig. 4.25 The theoretical prediction for the thickness of cartilage removed and the measured values of the vertical displacement.

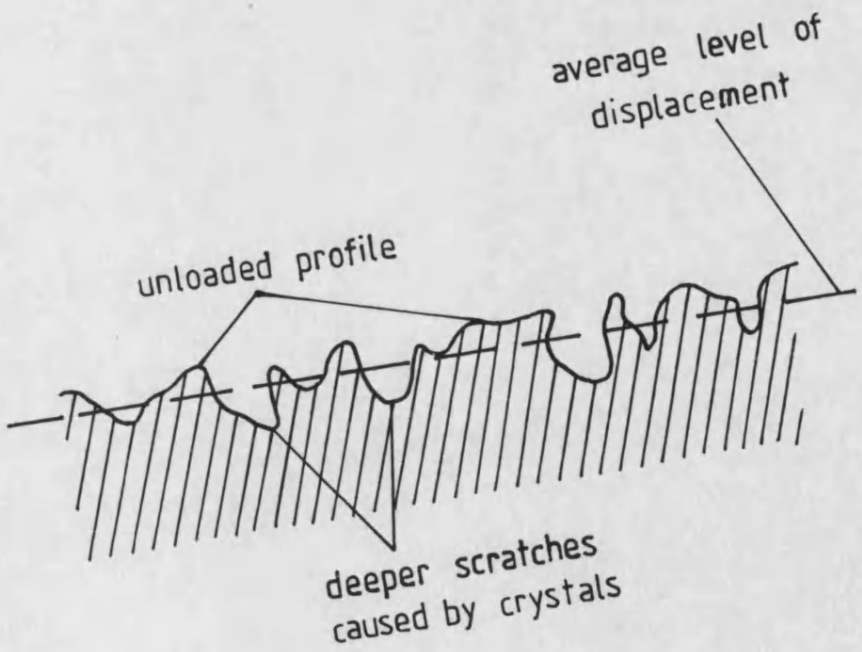


Fig. 4.26 A schematic diagram illustrating how deep scratches on the cartilage surface may remain undetected by the LVDT.

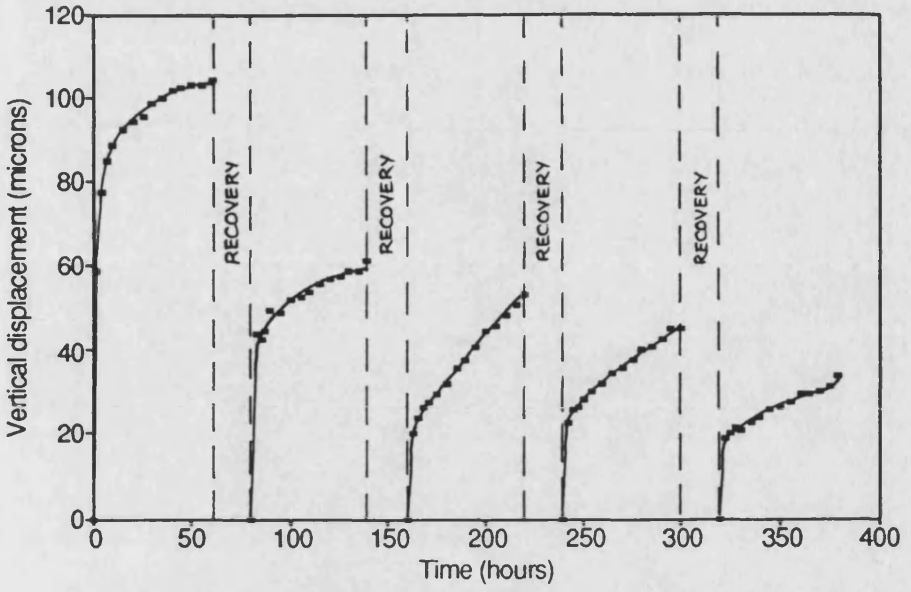


Fig. 4.27a The vertical displacement of articular cartilage during hourly intervals of a wear test.

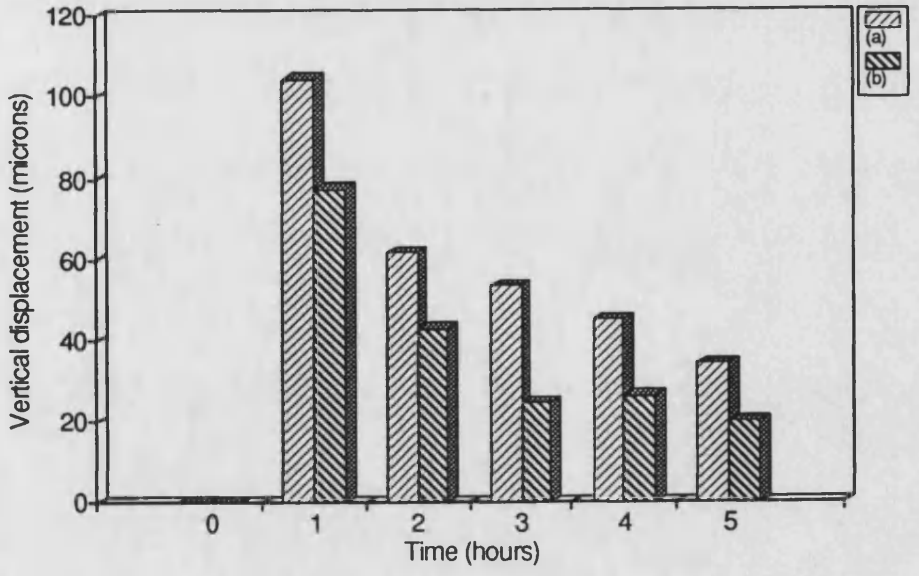


Fig. 4.27b The final vertical displacement (a) and the five minute creep component (b) of articular cartilage at hourly intervals.

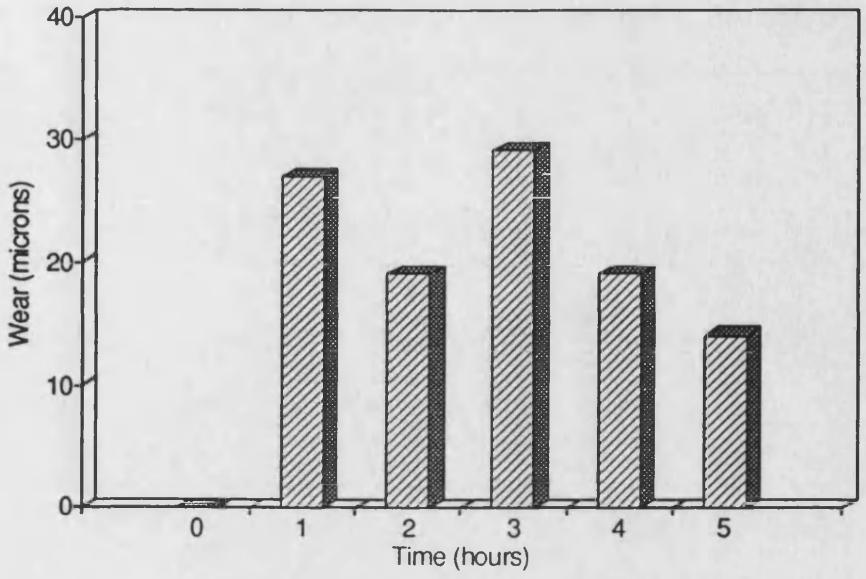


Fig. 4.27c The final vertical displacement minus the creep component at hourly intervals.

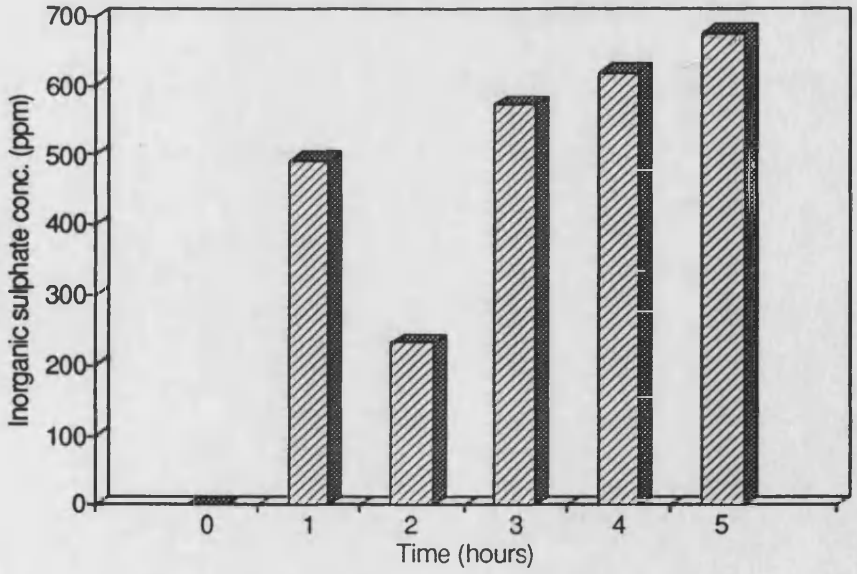


Fig. 4.28 The inorganic sulphate concentration measured in the lubricant at hourly intervals.

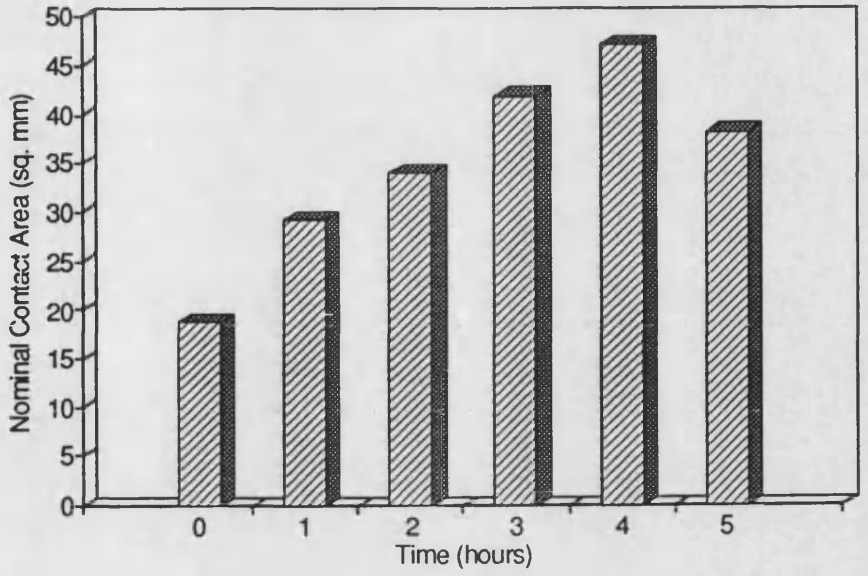


Fig. 4.29 The contact area measured at hourly intervals.

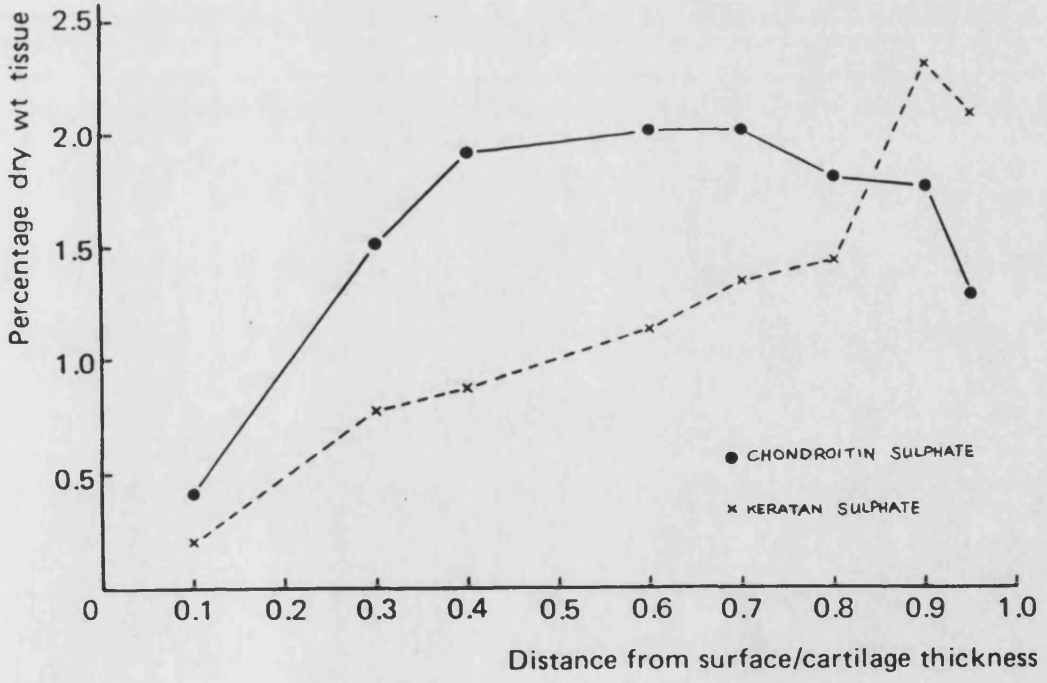


Fig. 4.30 The concentration of chondroitin and keratan sulphate with depth through the cartilage layer. (After Meachim & Stockwell, 1979.)

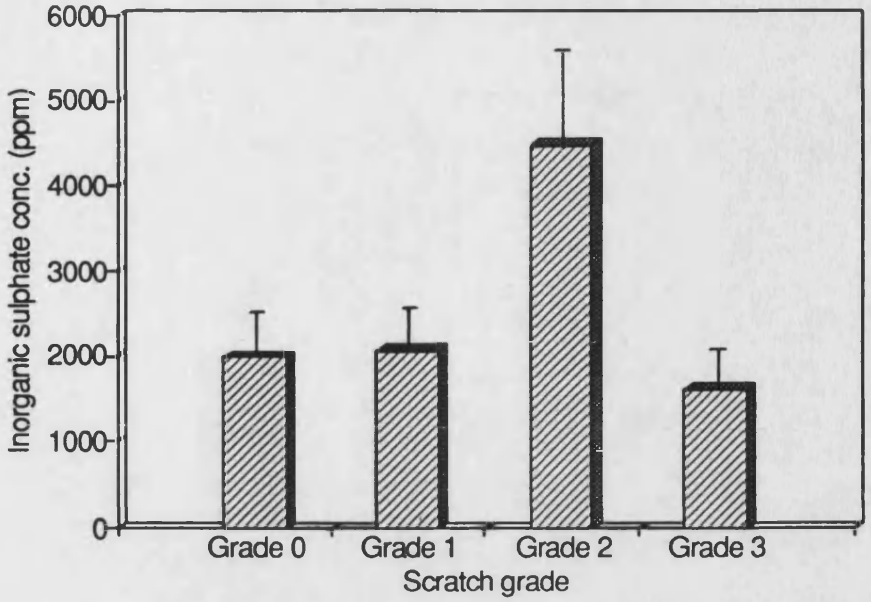


Fig. 4.31 The wear of articular cartilage scratched *in vivo*. Grade 0 indicated no visible damage to the cartilage surface and grade 3 indicated the presence of gross scratch marks on the cartilage surface.

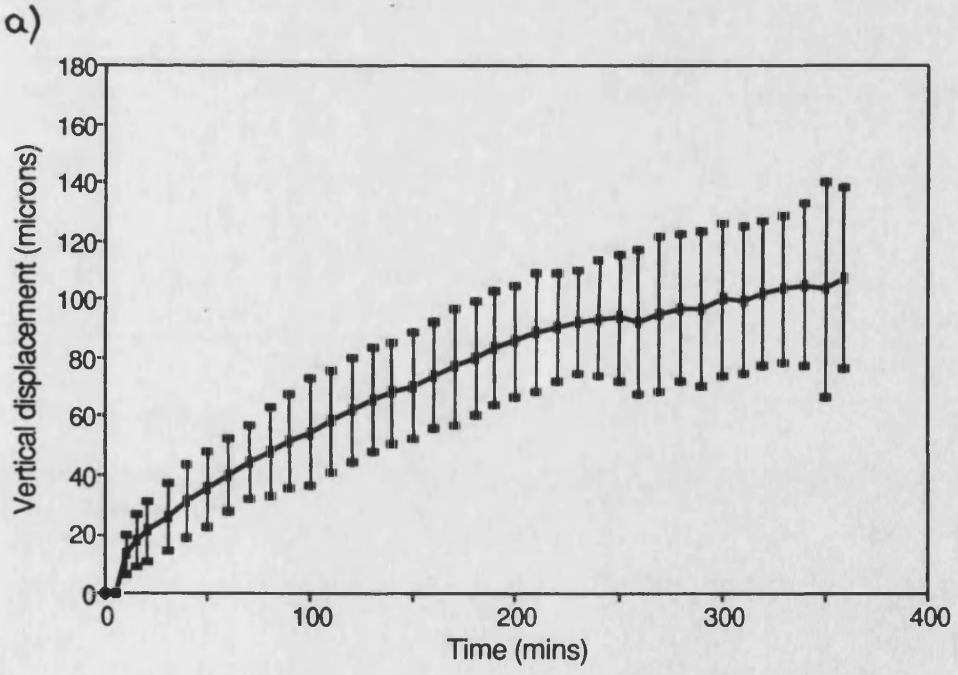


Fig. 4.32 The mean vertical displacement of a) grade 1 scratched cartilage during a wear test.

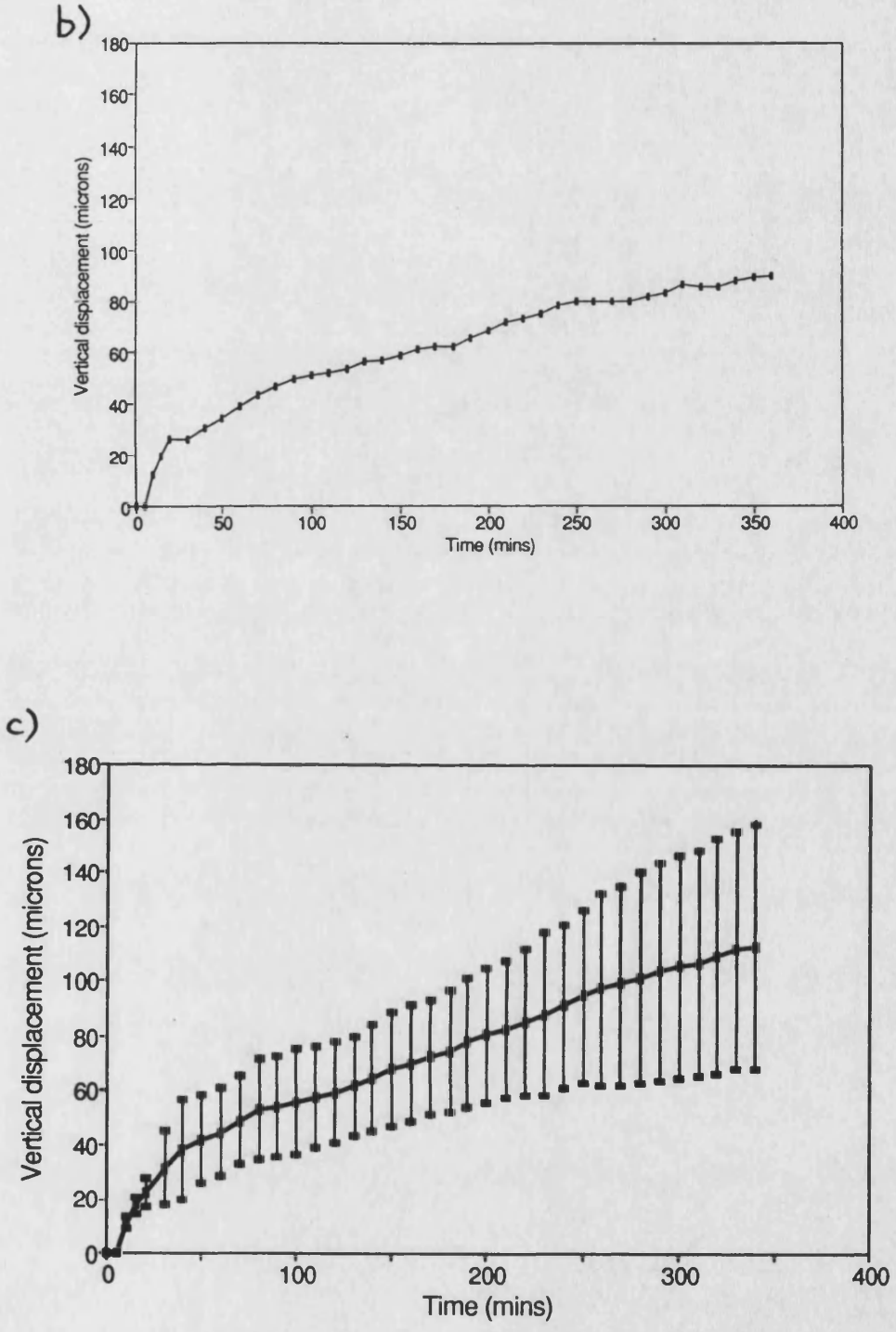


Fig. 4.32 The mean vertical displacement of b) grade 2 and c) grade 3 scratched cartilage during a wear test.

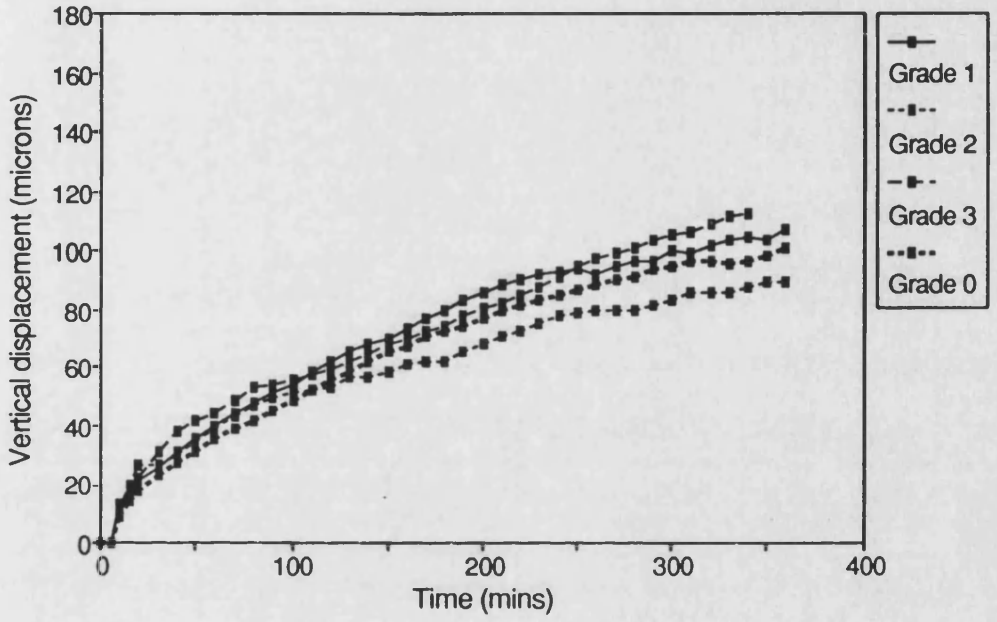


Fig. 4.33 The vertical displacement of normal articular cartilage and cartilage scratched *in vivo* during a wear test.

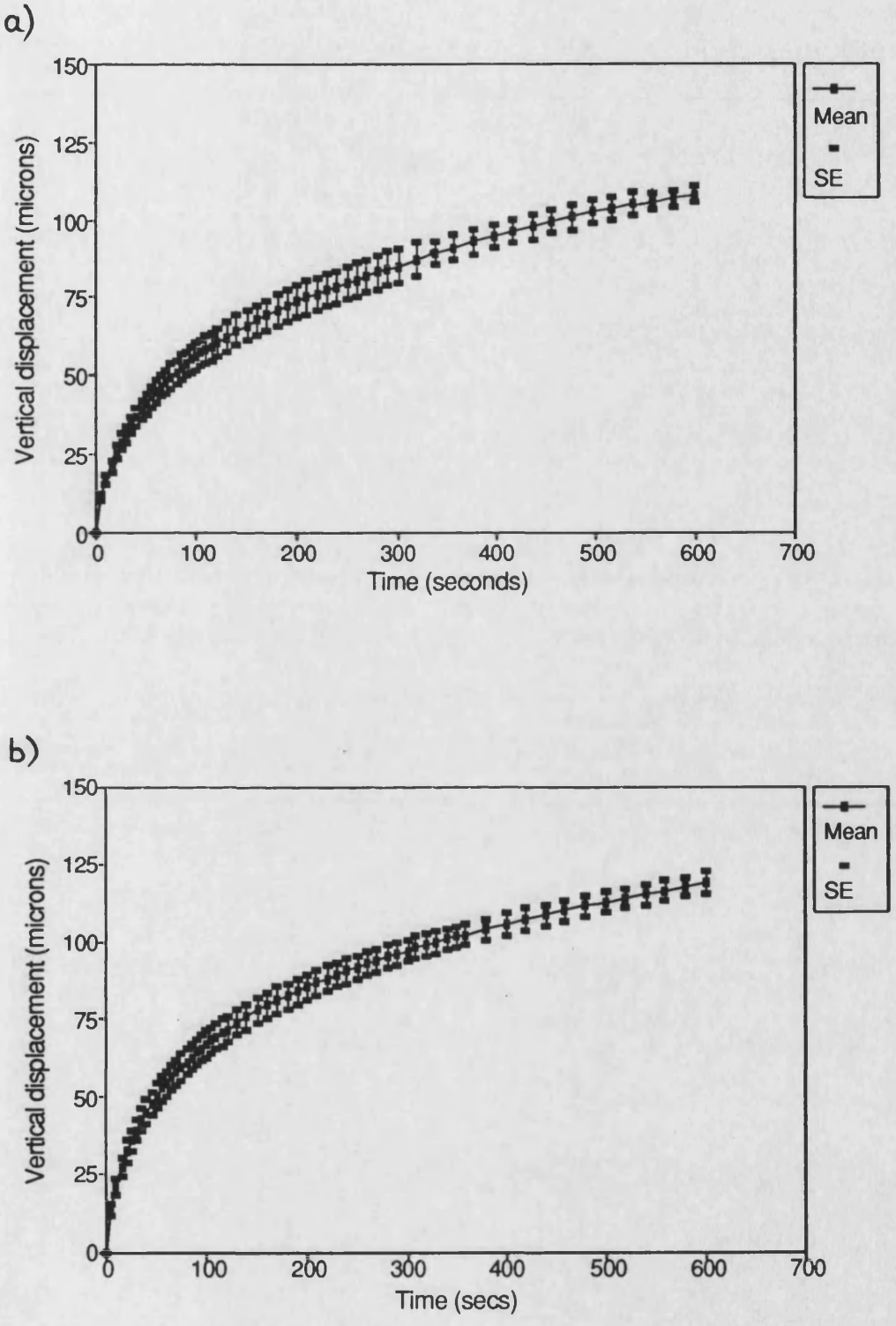
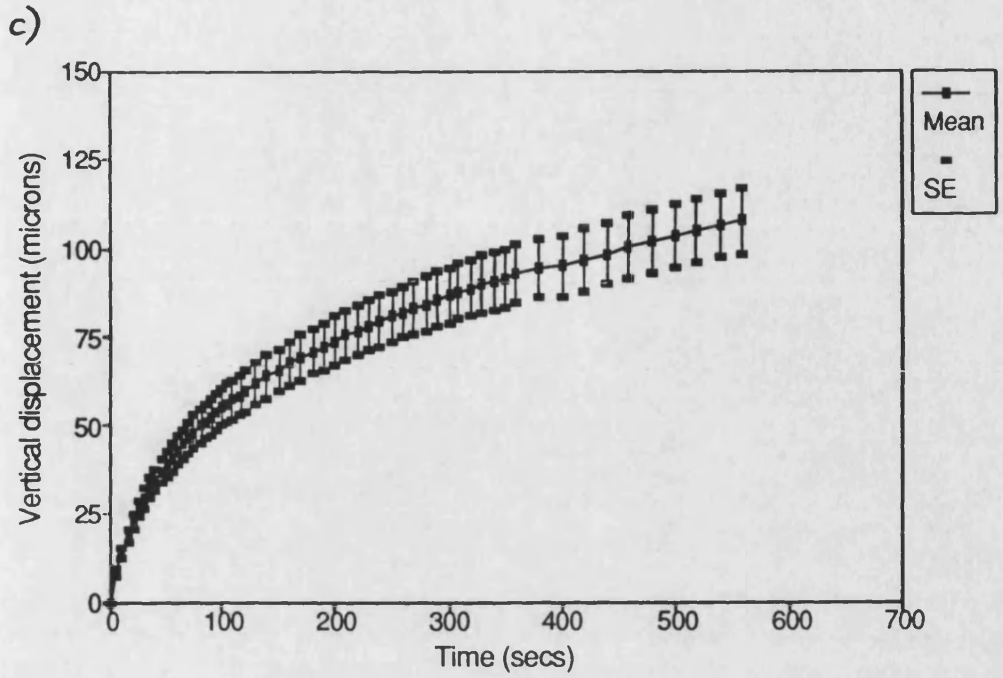


Fig. 4.34 The creep response for a) normal, b) grade 1 cartilage.



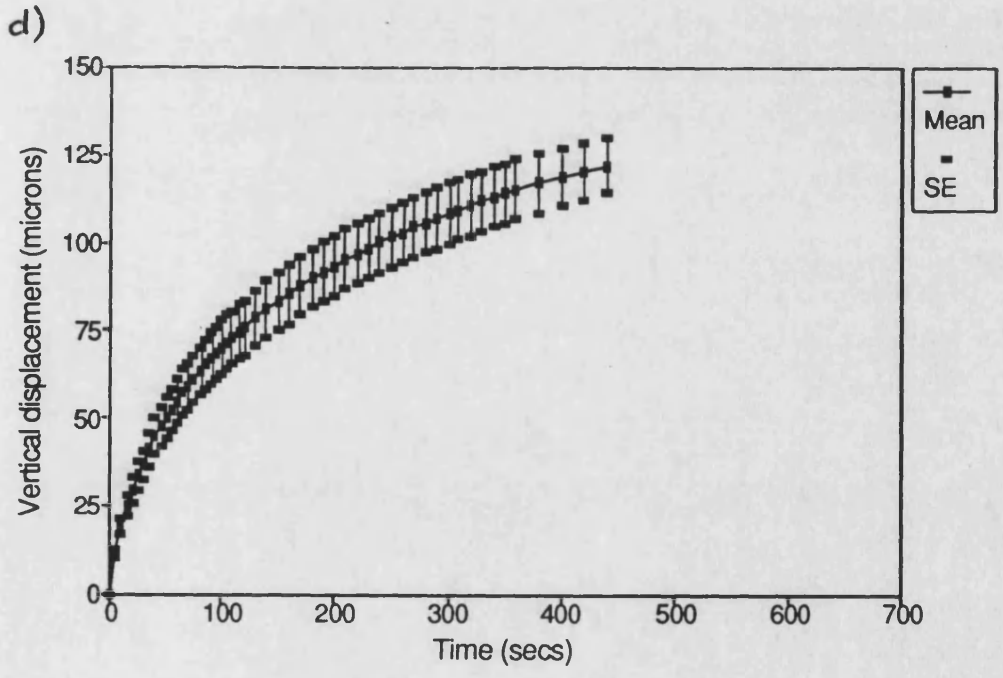


Fig. 4.34 The creep response for c) grade 2 and d) grade 3 cartilage.

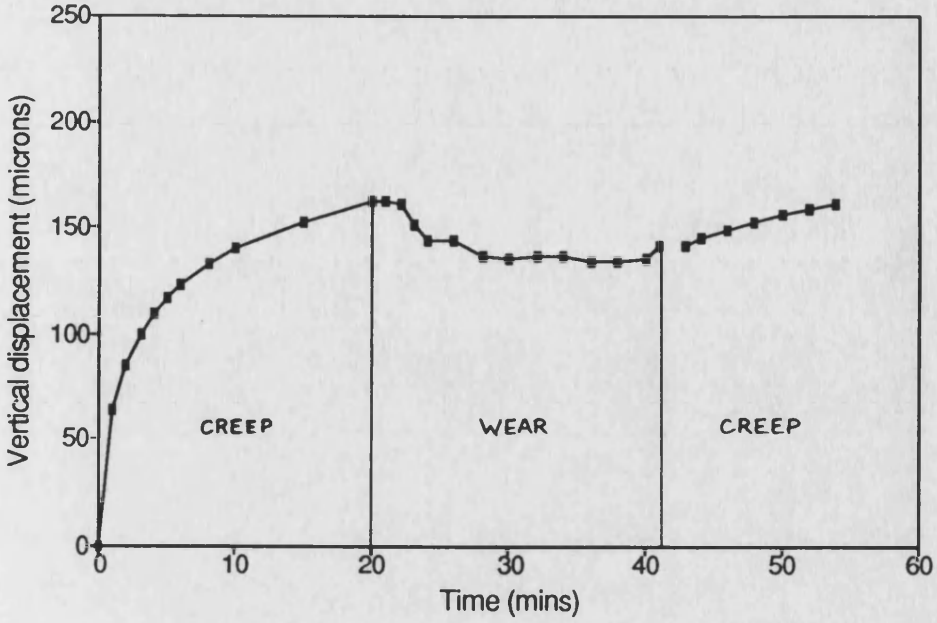


Fig. 4.35 The creep/wear/creep response of normal articular cartilage over one hour.

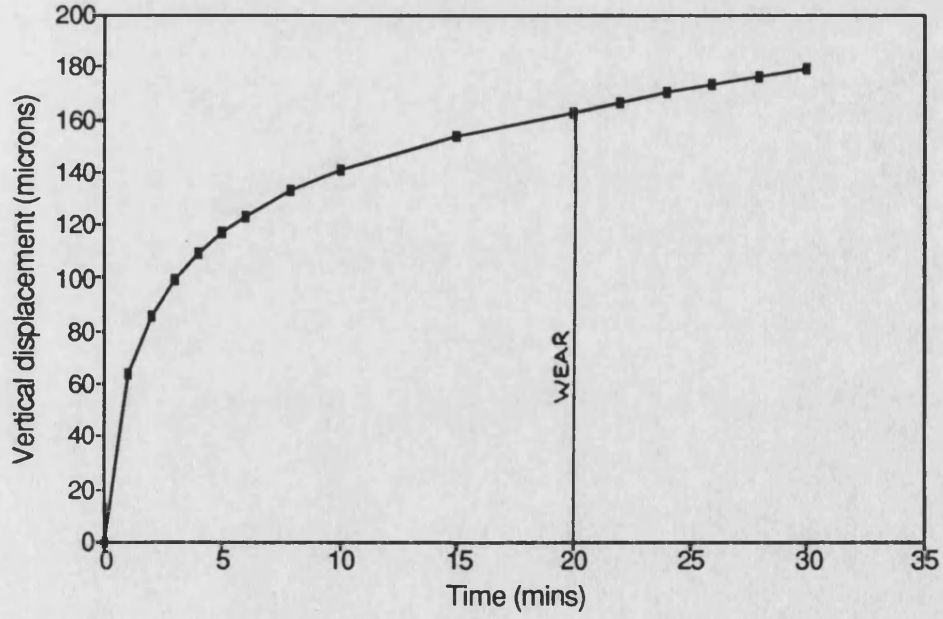


Fig. 4.36 The continuation of the creep response after a twenty minute wear period.

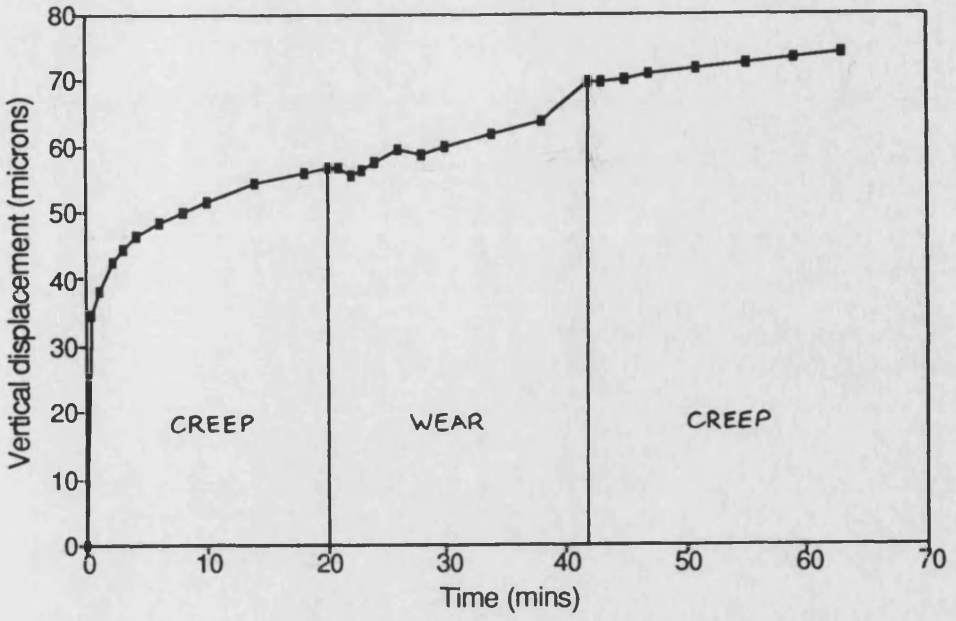


Fig. 4.37 The creep/wear/creep response of ultra-high molecular weight polyethylene.

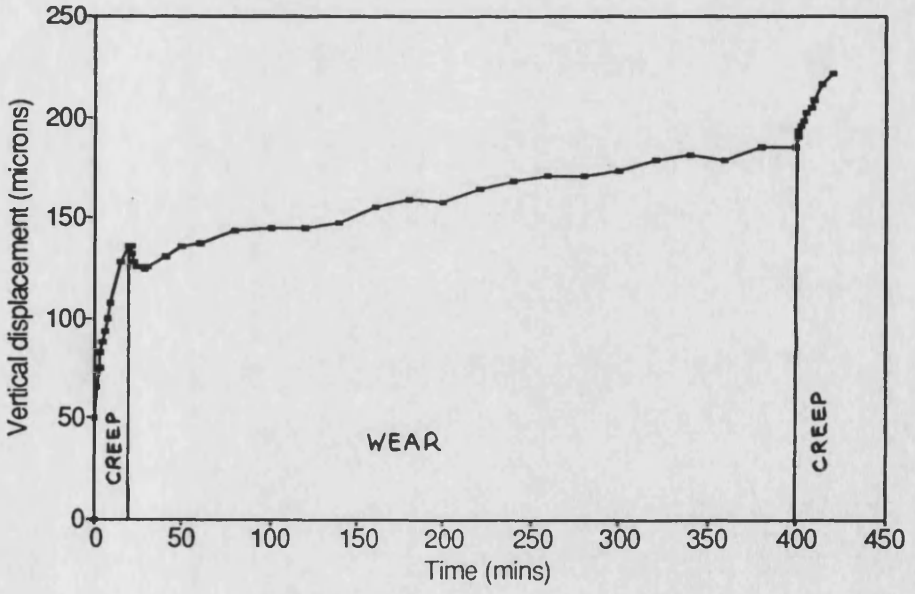


Fig. 4.38 The creep/wear/creep response of articular cartilage with a six hour wear period.

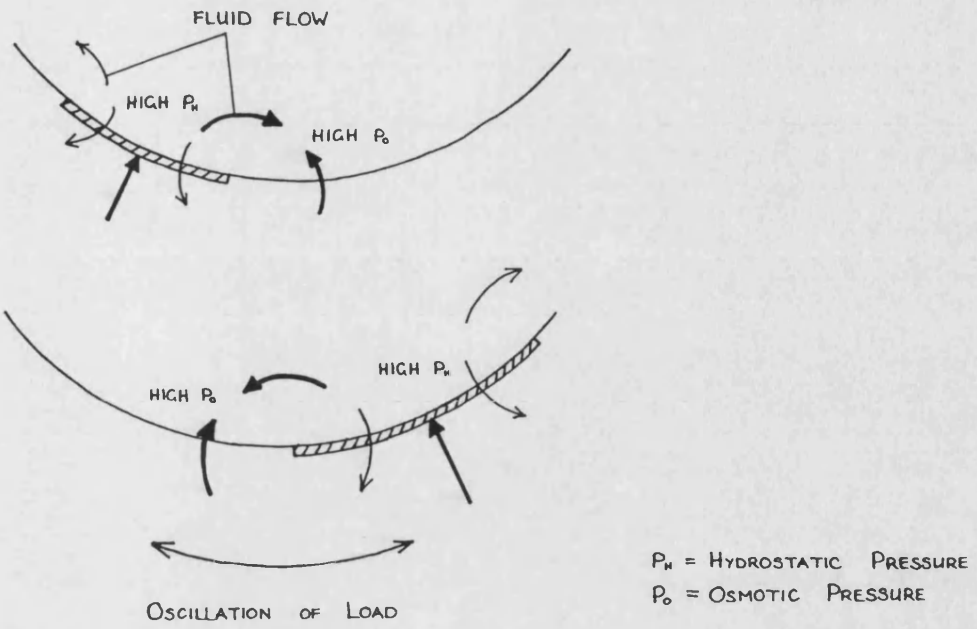


Fig. 4.39 A schematic diagram illustrating the possible generation of an internal "pump" created by differences in the hydrostatic and osmotic pressures in an oscillating joint.

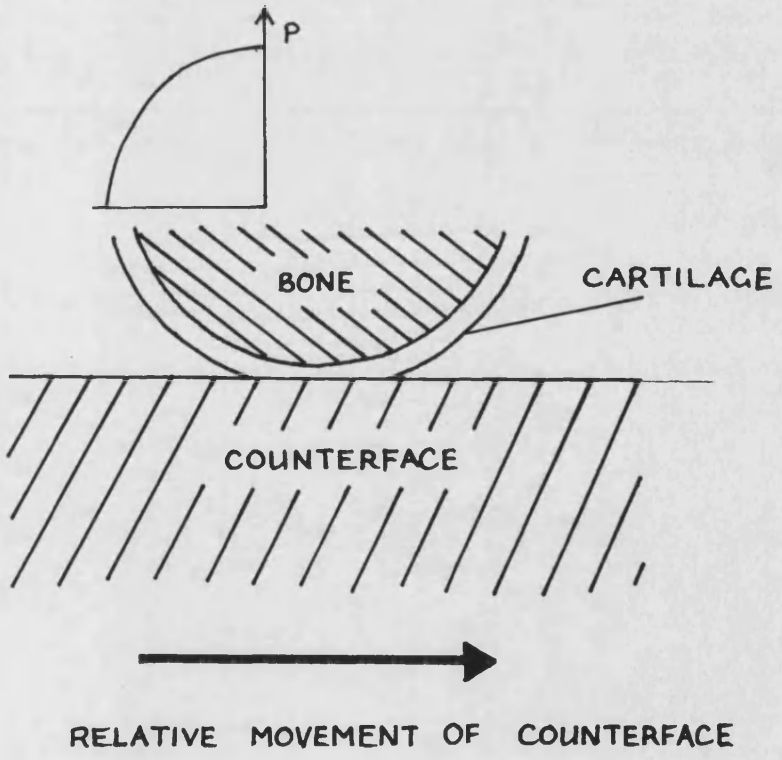


Fig. 4.40 A schematic diagram illustrating the hydrodynamic pressure set up within the lubricant as it approached the gap/contact area between the cartilage and the counterface.

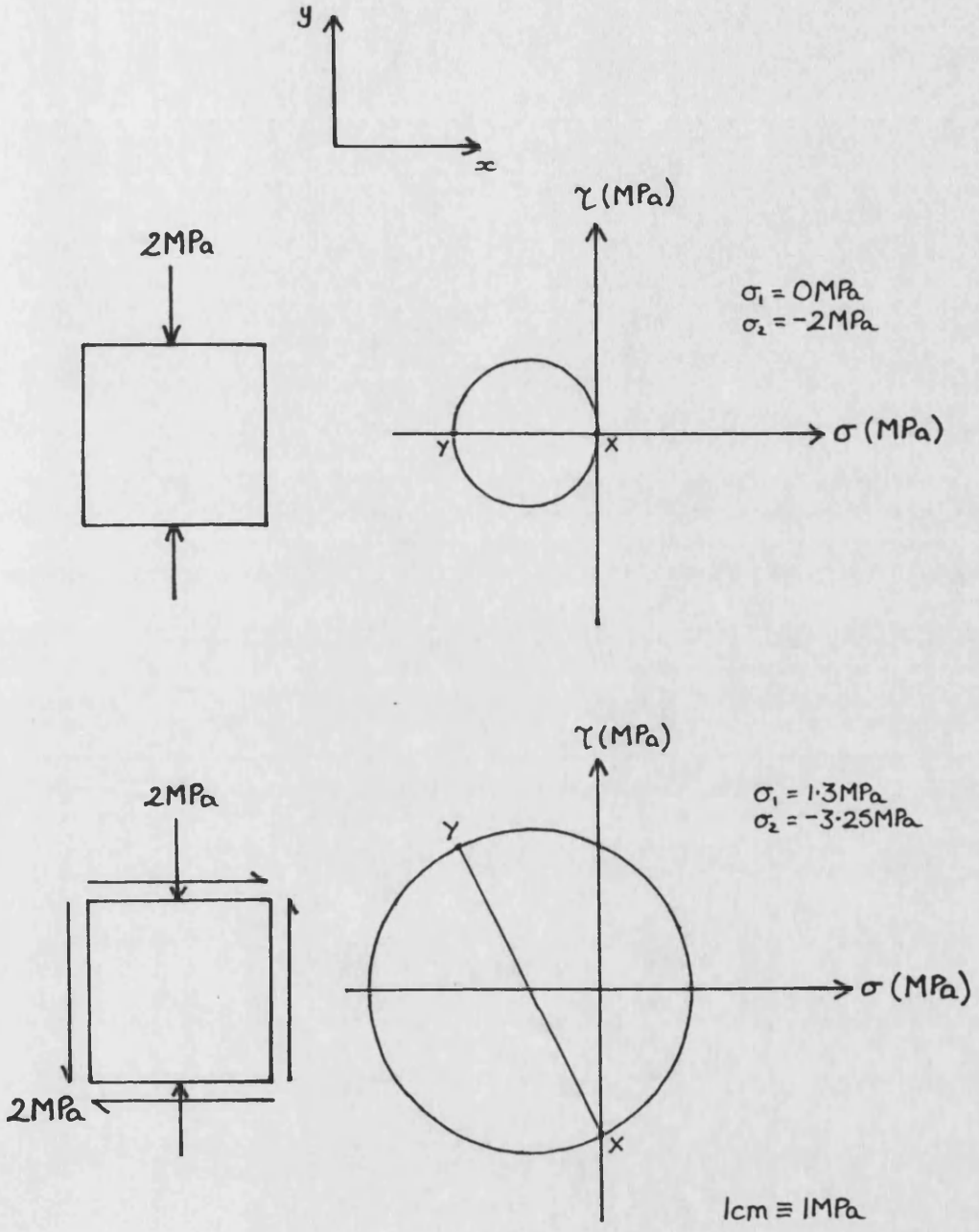


Fig. 4.41 Mohr's circle showing the maximum stress concentration within a material when subjected to shear forces in addition to compressive loading. A coefficient of friction of one has been assumed.

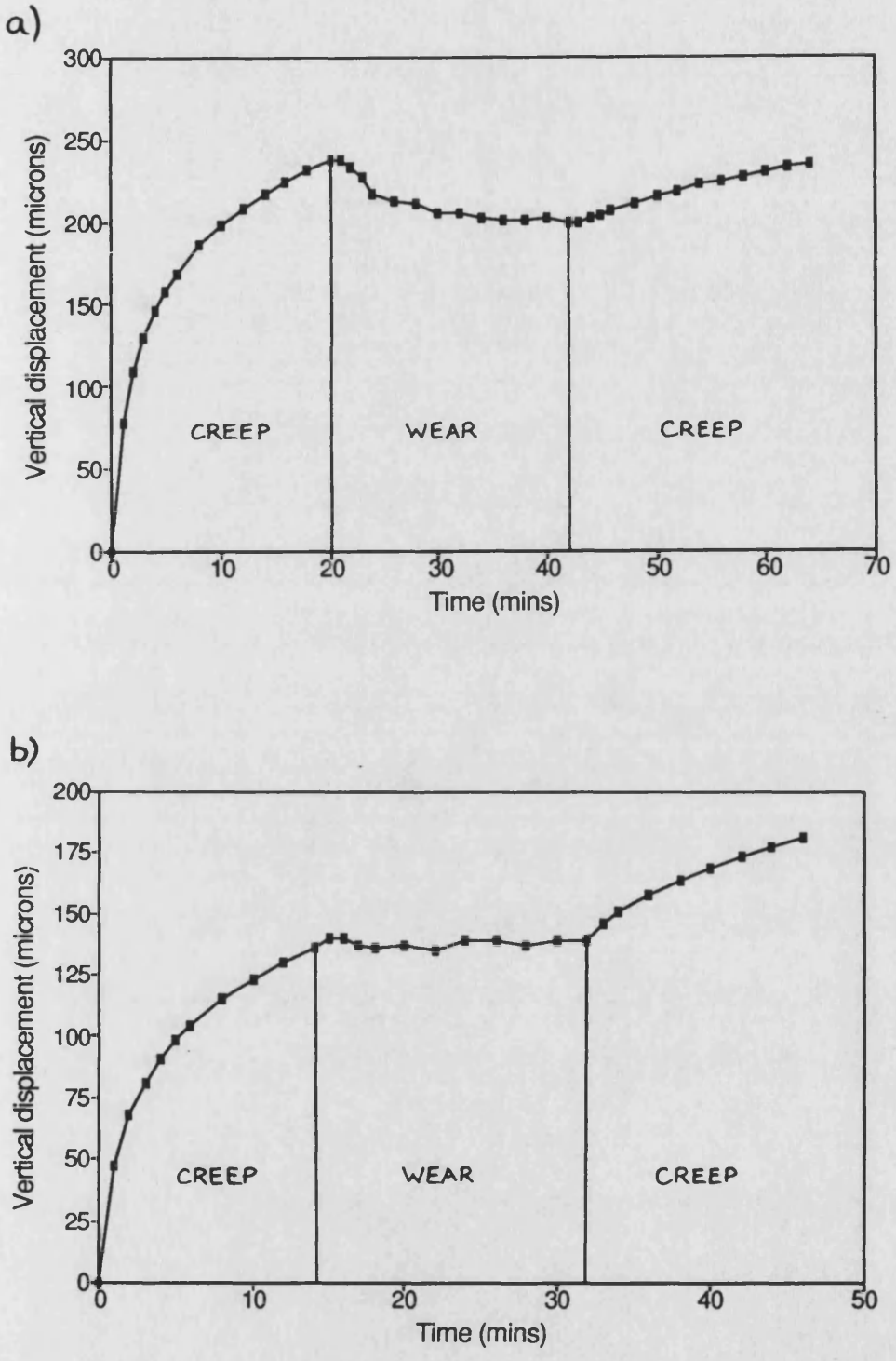


Fig. 4.42 The creep/wear/creep response for articular cartilage (a) initially worn for twenty minutes and (b) worn for a further fifteen minutes.

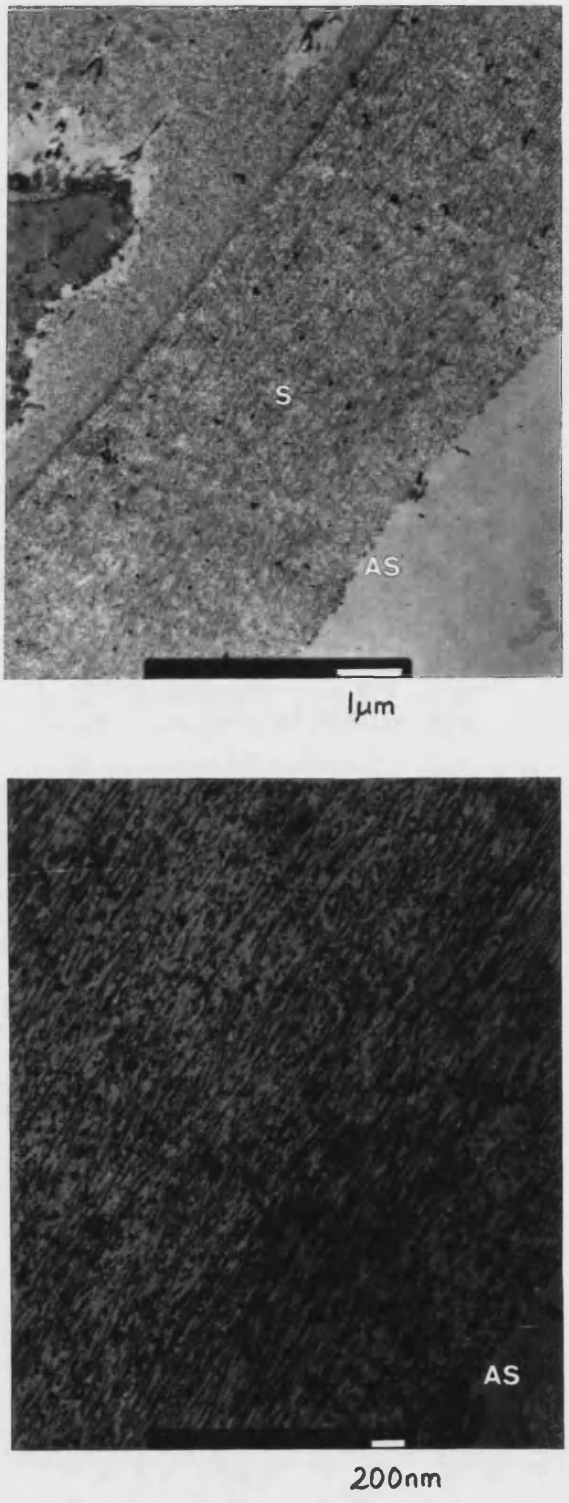


Fig. 4.43 TEMs of the superficial zone (S) of equine articular cartilage showing the predominantly parallel orientation of the collagen fibres. (AS = articular surface).

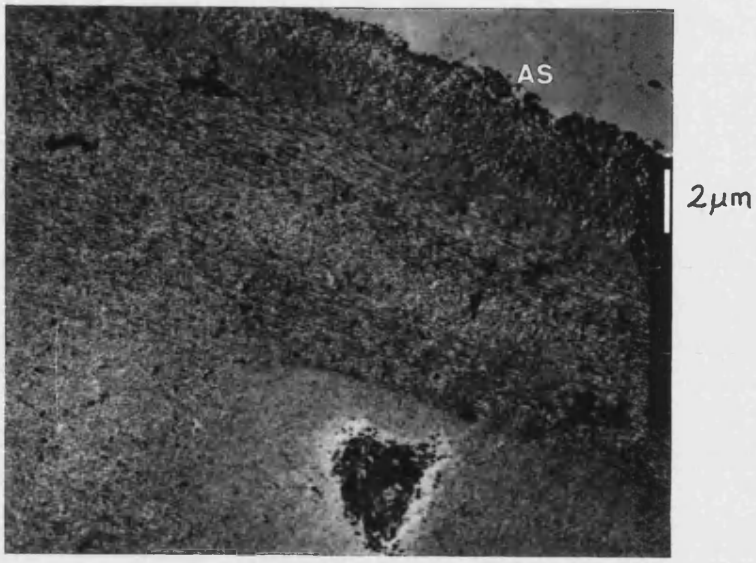


Fig. 4.44 TEM showing evidence of a composite layering of the collagen fibres. (AS = articular surface).

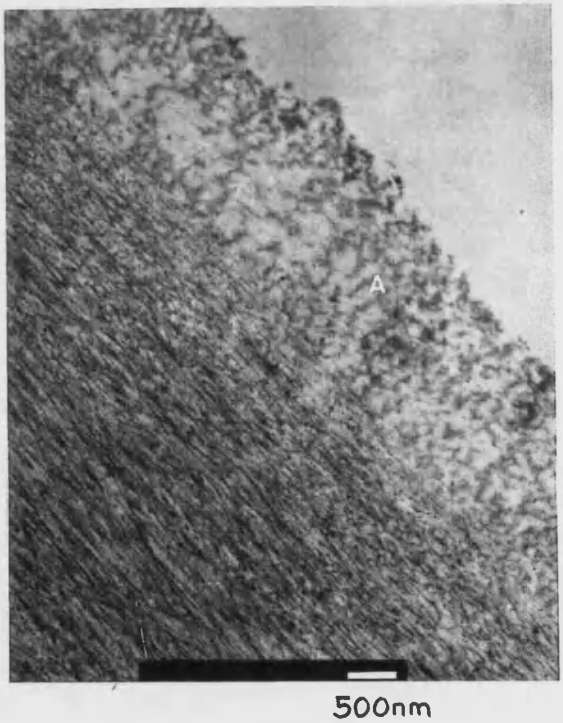


Fig. 4.45 TEM showing an amorphous layer (A) on the articular surface assumed to be the hyaluronate residue from the synovial fluid.

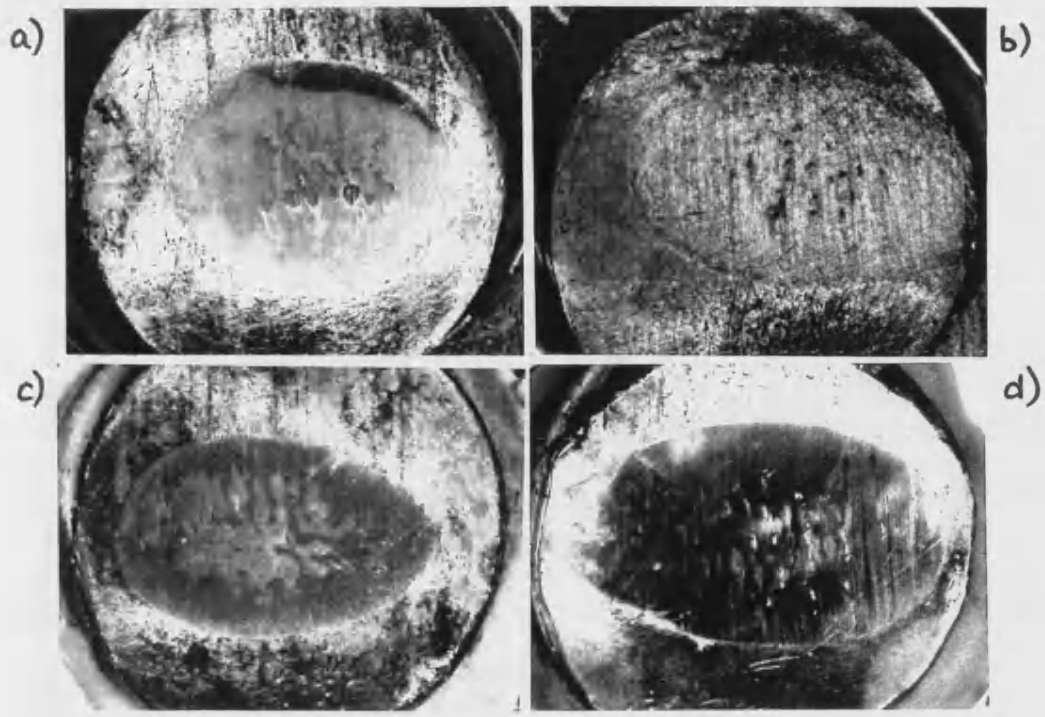


Fig. 4.46 Optical micrographs (OMs) of worn cartilage surfaces: a) no crystals, b) o-CPPT, c) m-CPPD and d) HA crystals present in the lubricant.

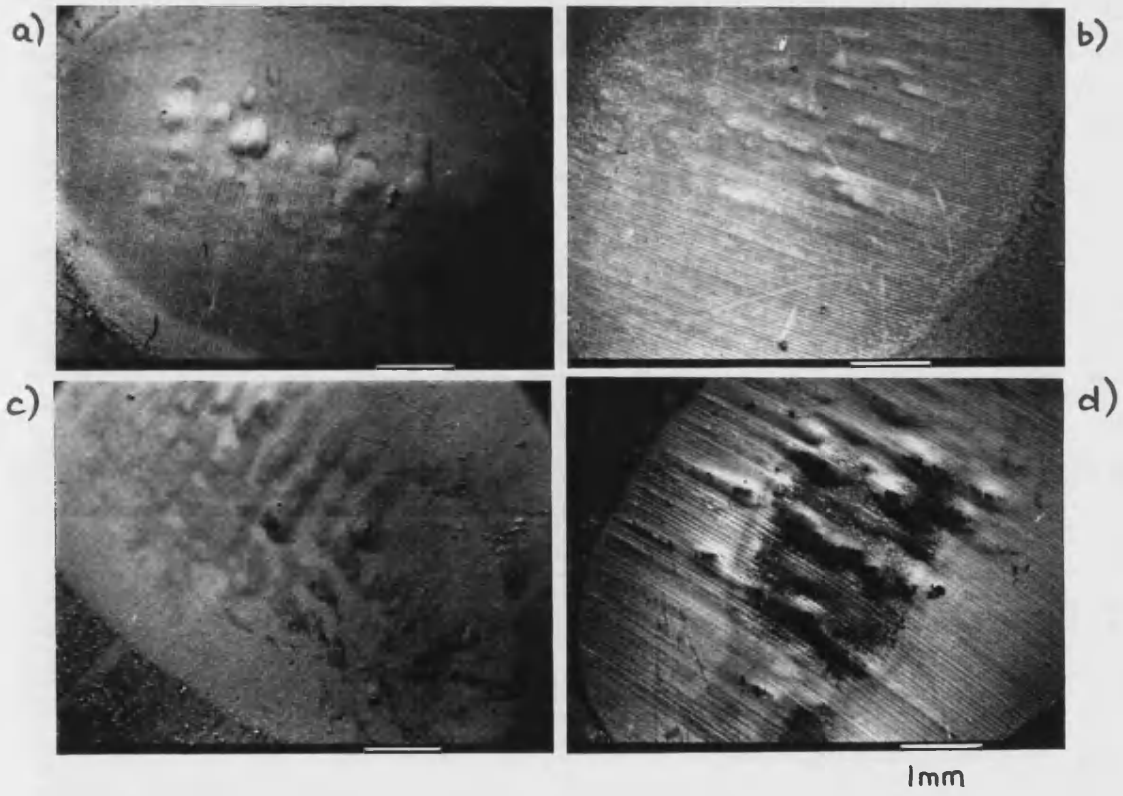


Fig. 4.47 SEMs of the contact areas: a) no crystals, b) o-CPPT, c) m-CPPD and d) HA crystals present in the lubricant.

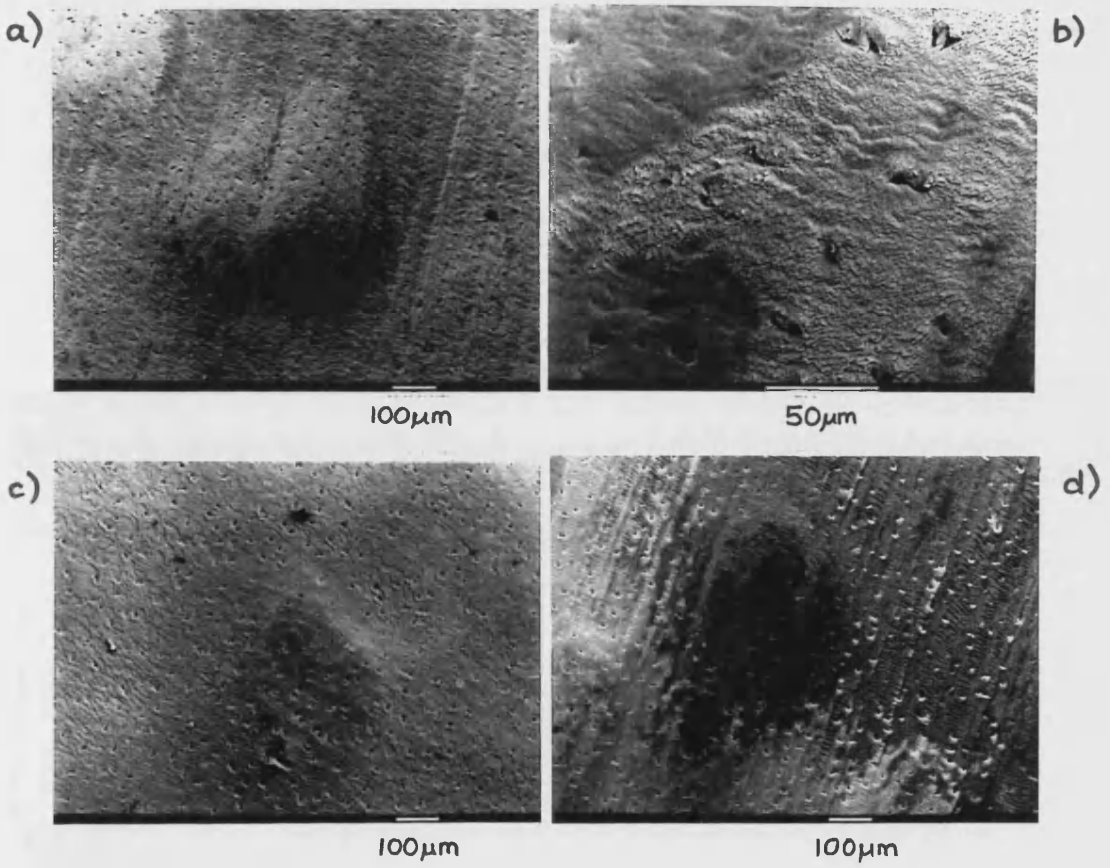


Fig. 4.48 SEMs of the raised blisters within the contact areas: a) no crystals, b) o-CPPT, c) m-CPPD and d) HA crystals present in the lubricant.

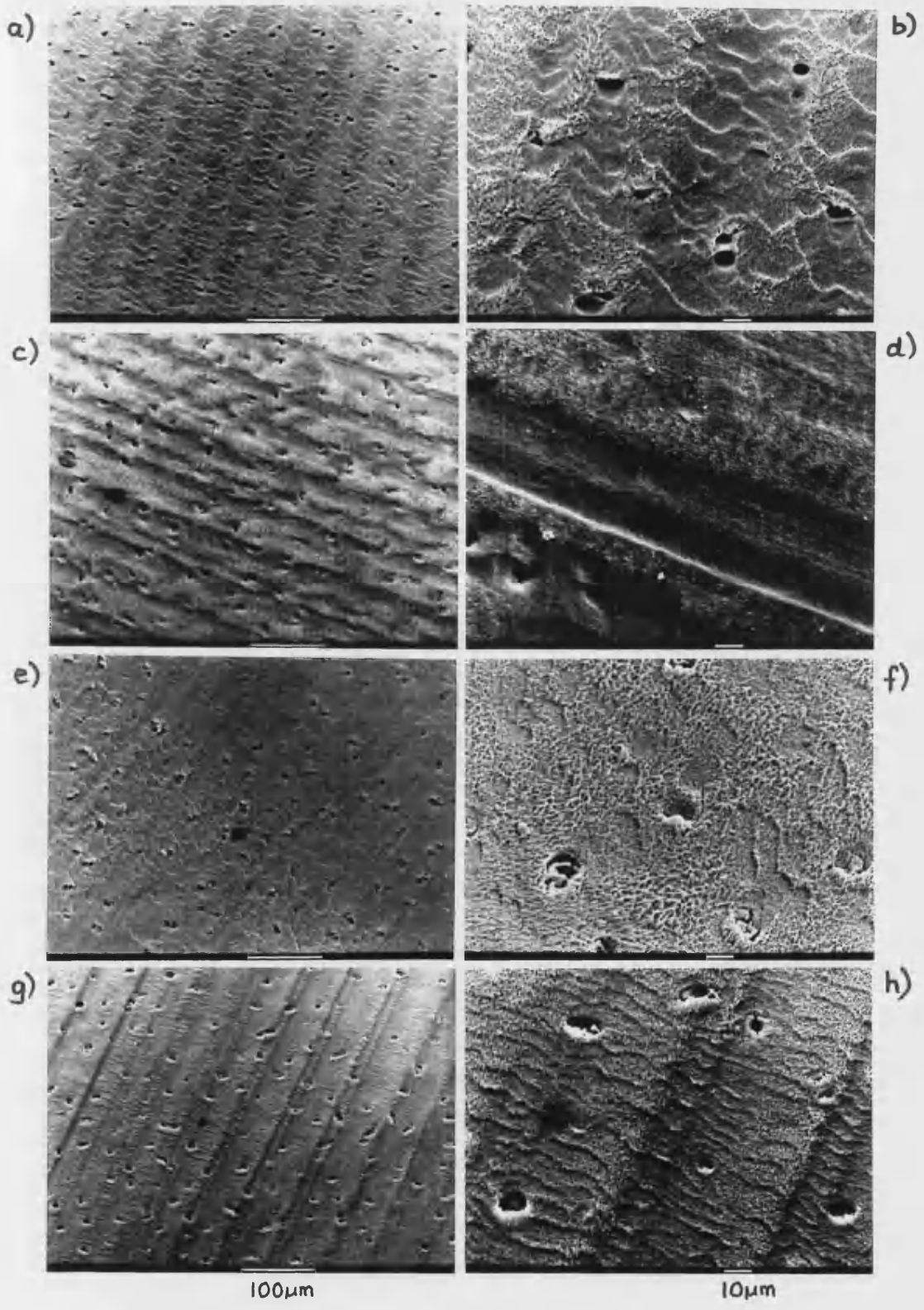


Fig. 4.49 SEMs illustrating the parallel scratch marks produced in the contact areas: a) and b) no crystals, c) and d) o-CPPT, e) and f) m-CPPD and g) and h) HA crystals present in the lubricant.

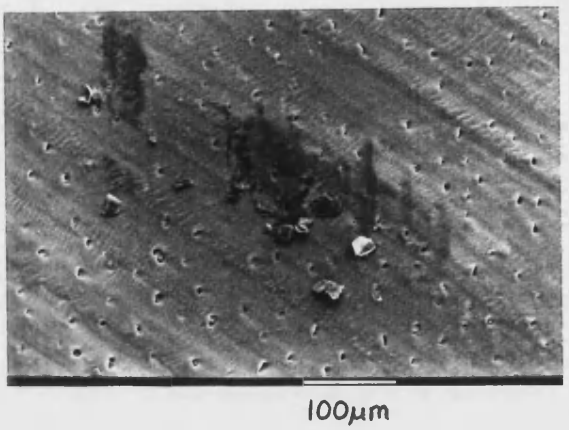


Fig. 4.50 Apparent crystal debris adjacent to shallow scratch marks.

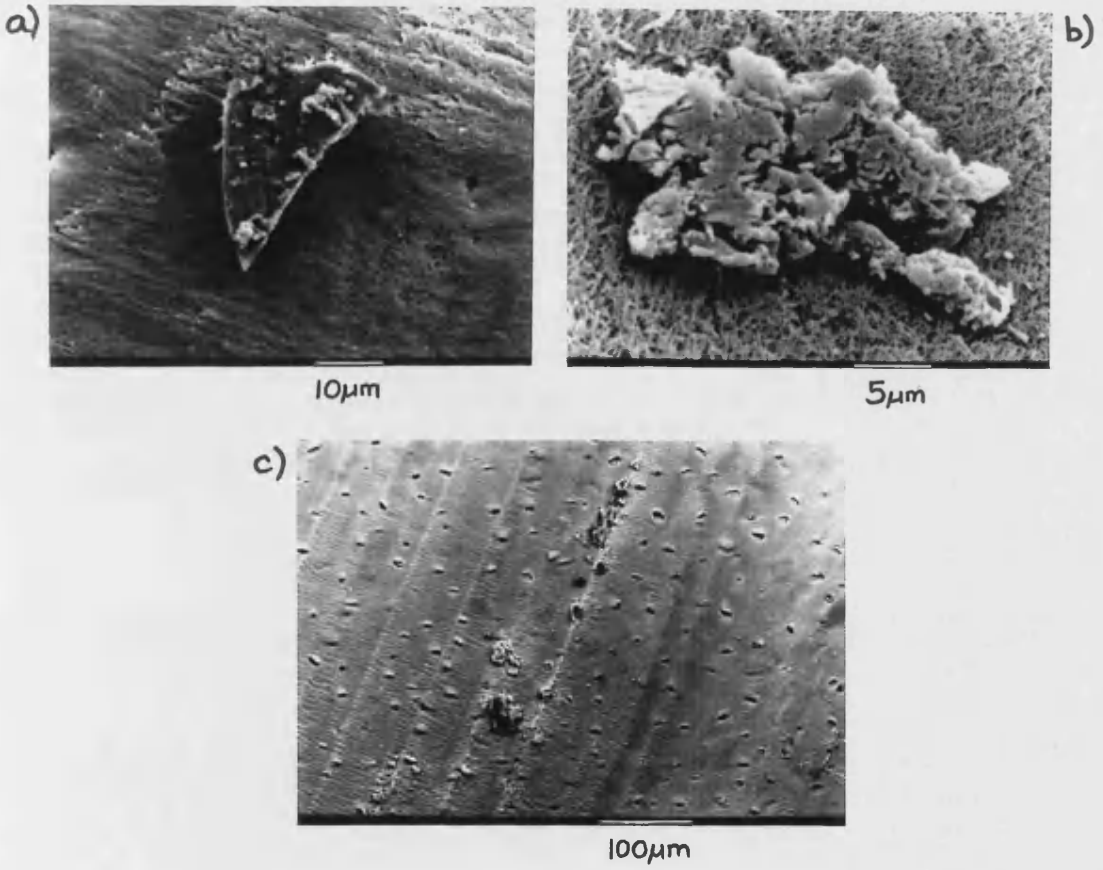


Fig. 4.51 Crystalline debris positively identified by EDA: a) o-CPPT, b) m-CPPD and c) HA crystals.

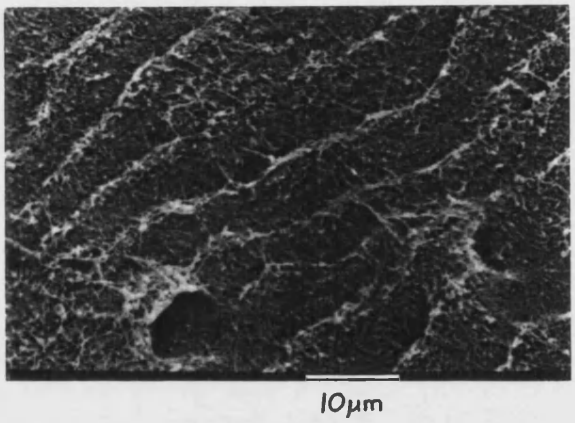


Fig. 4.52 SEM illustrating rucking of the cartilage surface with HA crystals present in the lubricant.

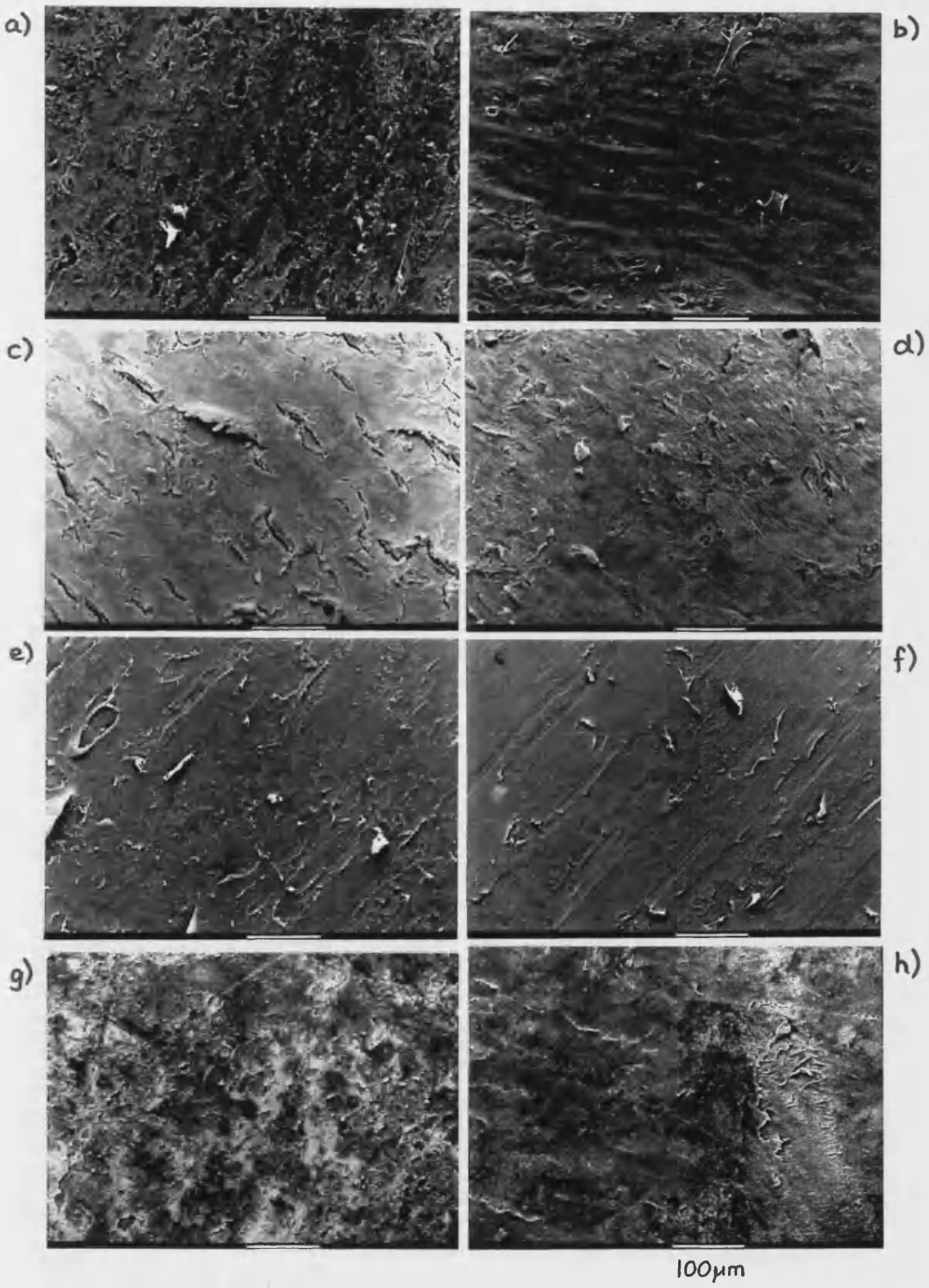


Fig. 4.53 SEMs of damaged cartilage on either side of the contact area: a) and b) no crystals, c) and d) o-CPPT, e) and f) m-CPPD and g) and h) HA crystals present in the lubricant.

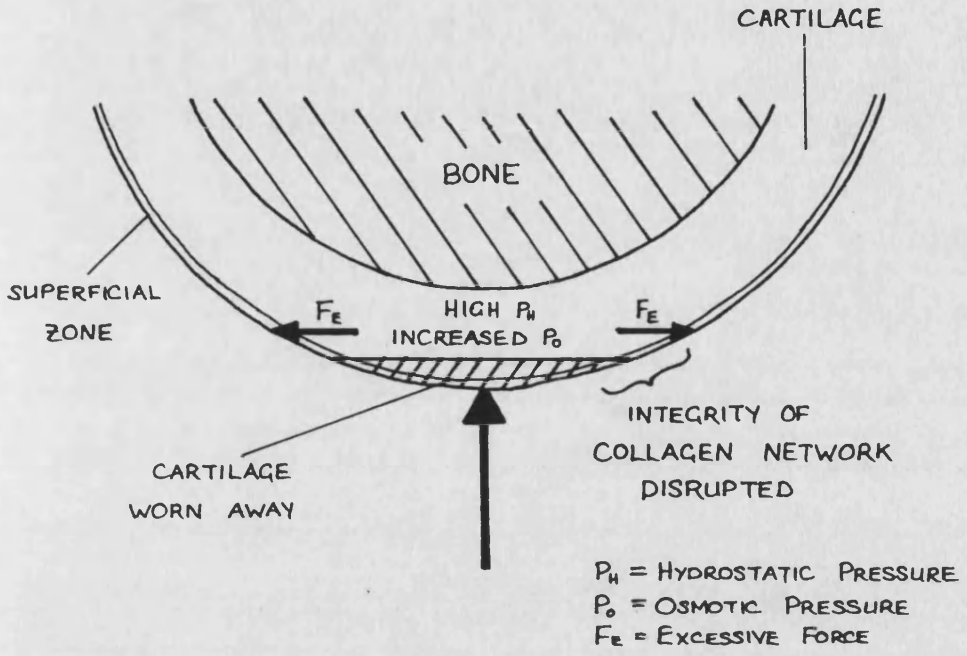


Fig. 4.54 A schematic diagram illustrating how the removal of part of the cartilage surface could disrupt the integrity of the cartilage structure as a whole.

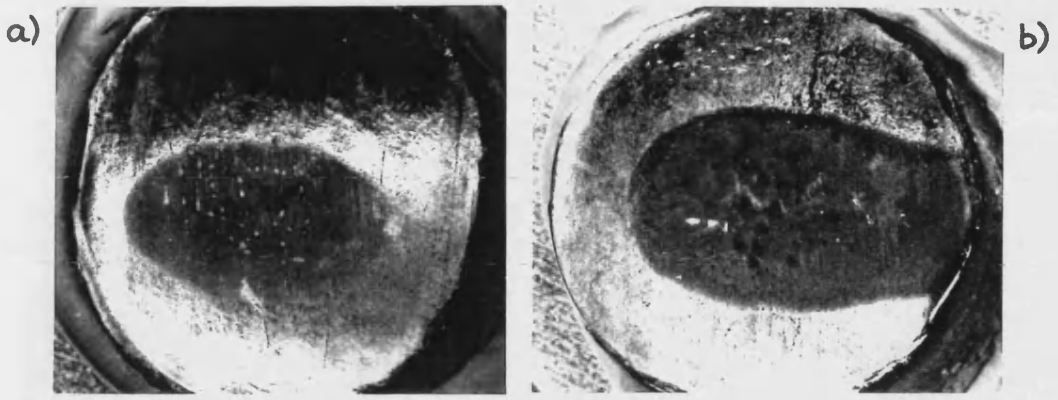


Fig. 4.55 OMs of articular cartilage worn under nominal contact stresses of a) 0.7MPa and b) 2.7MPa.

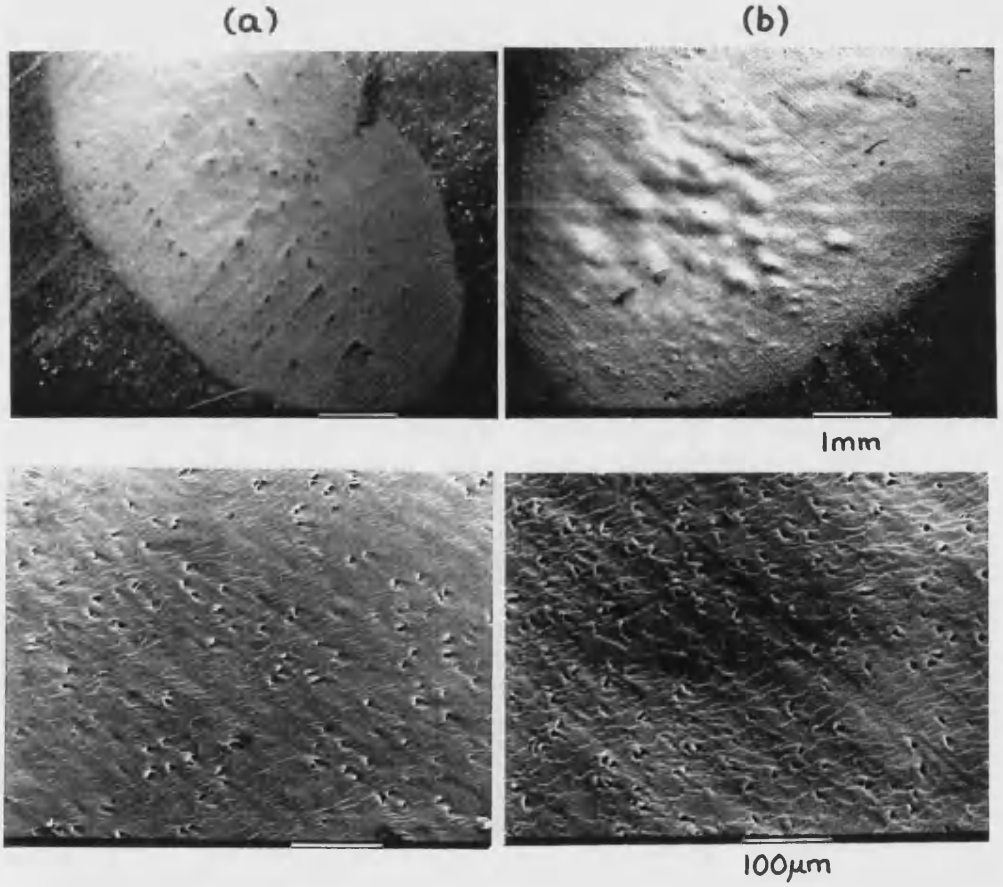


Fig. 4.56 SEMs of cartilage worn under nominal contact stresses of a) 0.7MPa and b) 2.7MPa.

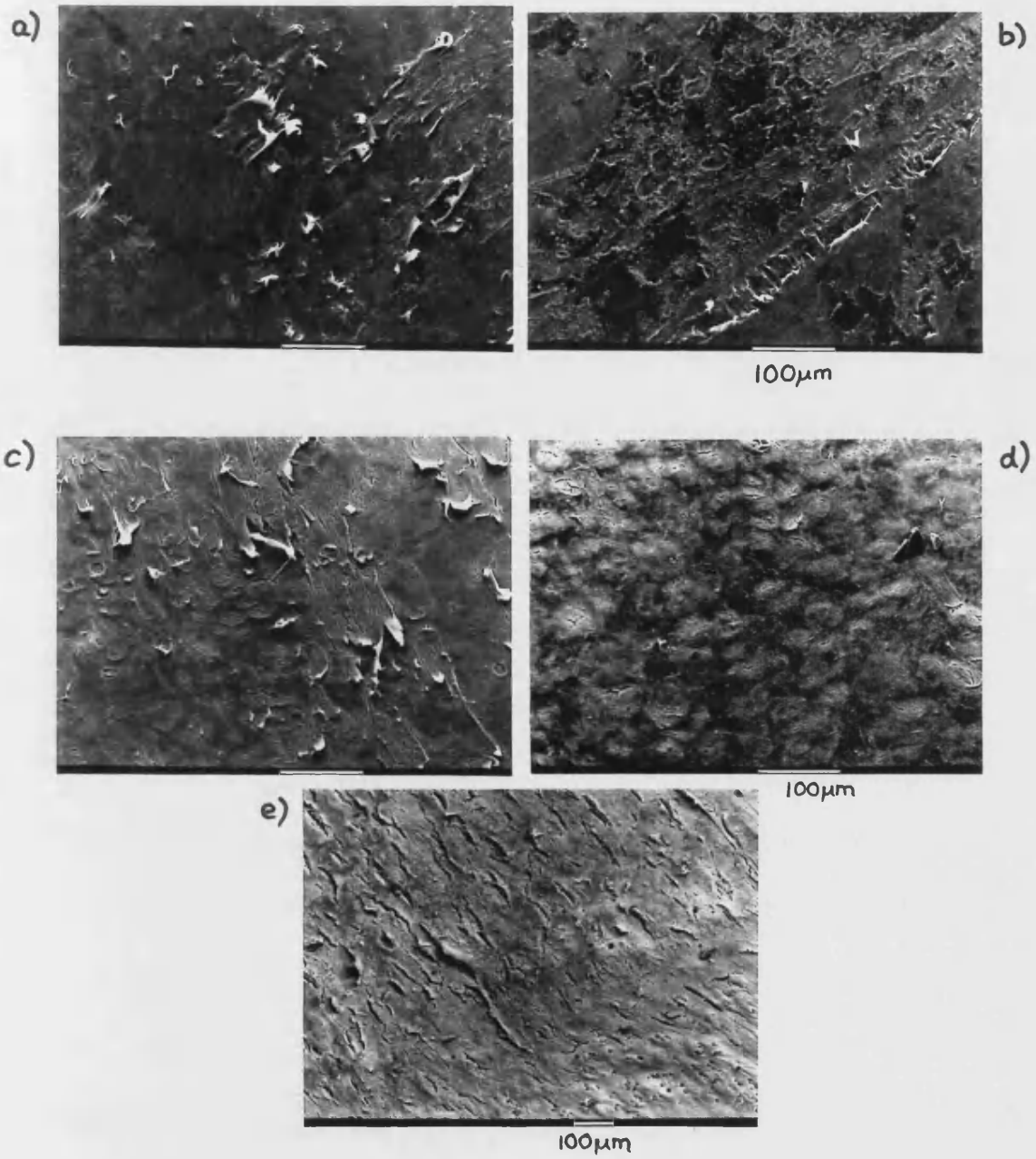


Fig. 4.57 SEMs of damaged cartilage on either side of the contact area: a) and b) for a nominal contact stress of 0.7MPA, and c), d) and e) for a nominal contact stress of 2.7MPa.

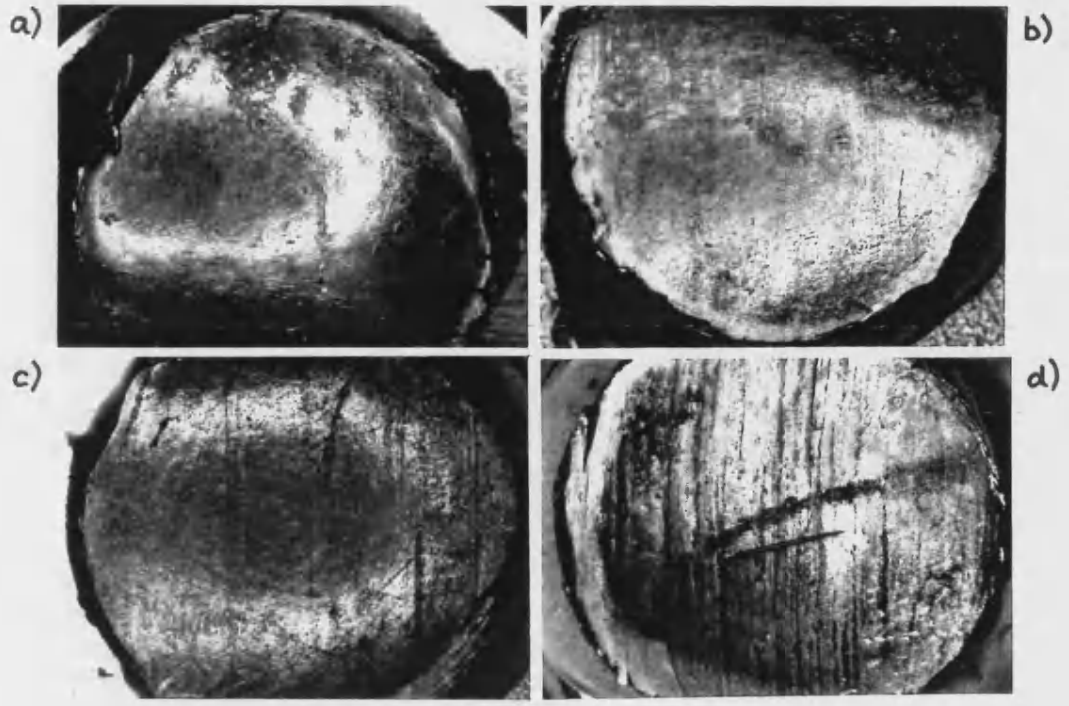


Fig. 4.58 OMs of cartilage scratched *in vivo*: a) grade 0, b) grade 1, c) grade 2 and d) grade 3.

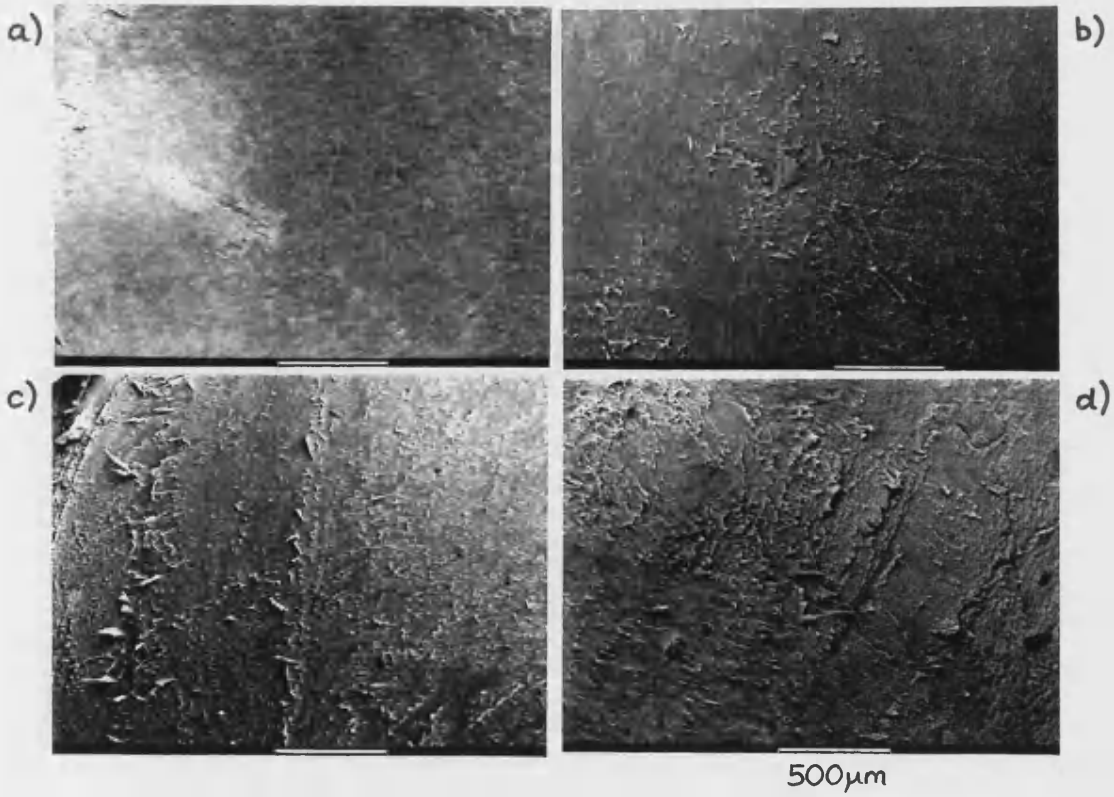


Fig. 4.59 SEMs of cartilage scratched *in vivo*: a) grade 0, b) grade 1, c) grade 2 and d) grade 3.

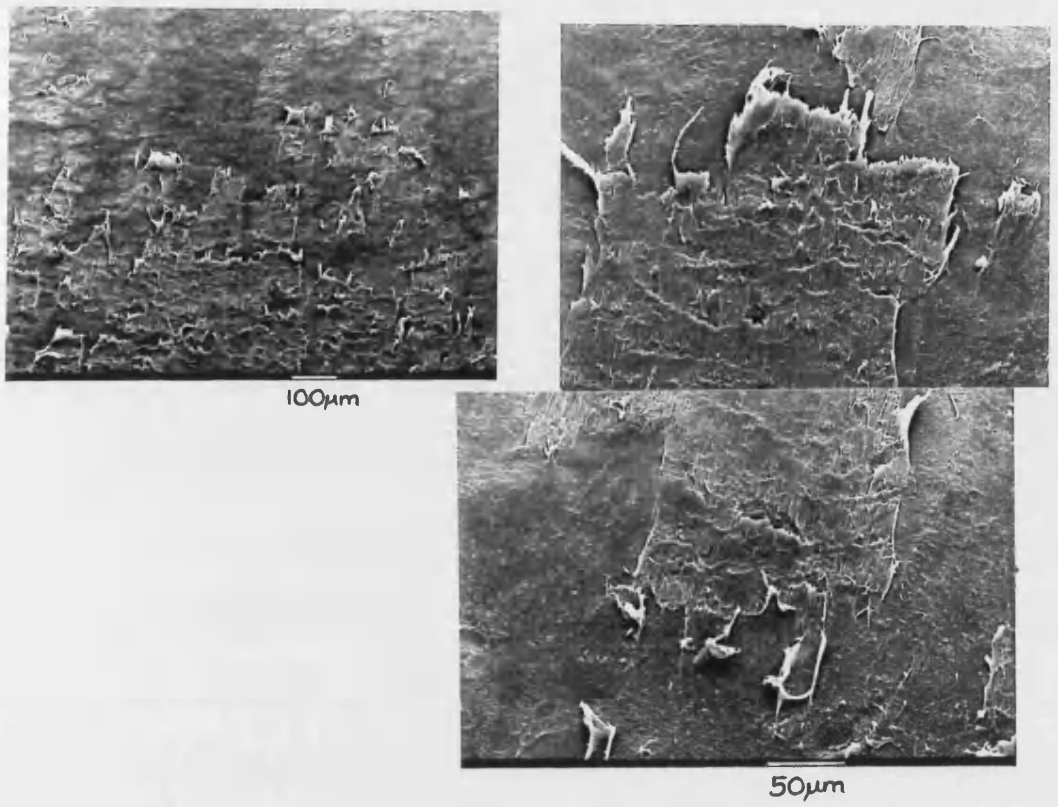


Fig. 4.60 SEM showing peeling of the surface of a grade 2 specimen.

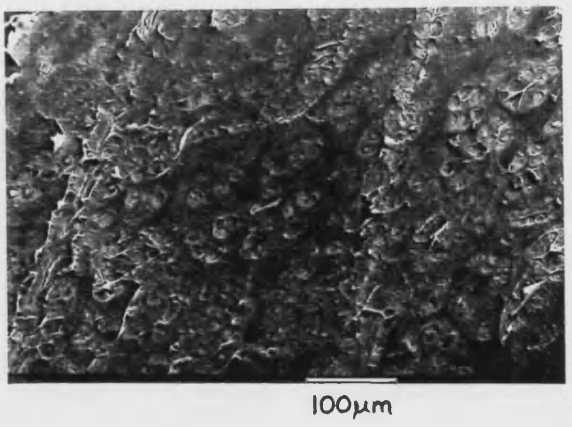


Fig. 4.61 SEM showing pitting of the surface of a grade 2 specimen.

5. CRYSTAL AGGREGATES IN ARTICULAR CARTILAGE

5.1 INTRODUCTION

Crystal deposits are known to occur commonly in elderly and osteoarthritic articular cartilage (section 2.5). It has been suggested (section 2.5.2) that the presence of large crystal deposits within the cartilage could alter the load carrying capability and thus have potentially damaging consequences for the structural integrity of the diseased tissue. This raises important questions with regard to the distribution of local stresses and strains induced in such tissue, and whether the onset of degeneration of the articular cartilage under such conditions could have a mechanical as well as a biochemical component, (section 2.5.2) (Sokoloff, 1969, Bollet, 1969, Anderson *et al*, 1991). Before an assessment of the stress concentrating effects of any crystal deposits could be made it would be necessary to have available data on their size and distribution within the cartilage matrix.

This study was undertaken in order to ascertain the gross morphology of large aggregates of crystals within articular cartilage, the distribution of these aggregates within the cartilage layer, the nature of the interface between the crystal aggregate and the cartilage matrix, the organisation of the individual

crystals within the aggregates, and their morphology. It is intended that the results will be used in conjunction with finite element analysis to investigate the stresses generated as a result of an aggregate, or a distribution of aggregates, being present within a cartilage matrix.

5.2 LITERATURE REVIEW

The first report of articular chondrocalcinosis (calcium pyrophosphate deposition disease) in the literature was by Zitnan and Sitaj (1963). Much of the subsequent early work on crystal deposits was based on radiography of linear calcific deposits concentrated in the midzone of the articular cartilage (Bundens *et al*, 1965, and Currey *et al*, 1966). However, the resolution of radiographs used for such purposes is known to be poor (Genant, 1976) and detailed analysis of the mineral deposits is not possible. Many workers, including Zitnan *et al* (1963), Bundens *et al* (1965) and Lagier & Baud (1968), used x-ray diffraction (XRD) to identify the composition of these crystal deposits. Zitnan *et al* (1963) identified the crystals as a carbonate apatite but could not be more specific. Bundens *et al* (1965) identified the crystals as calcium pyrophosphate dihydrate (CPPD), and Lagier and Baud (1968) further characterised them as triclinic CPPD, occassionally associated with the monoclinic form.

Histology, in conjunction with polarised light microscopy, is a standard method for the identification of crystal deposits within post-mortem articular cartilage (Bjelle, 1972, Reginato *et al*, 1974, Schumacher, 1976, Linden & Telhag, 1977, and Mitrovic, 1983). A recognised problem associated with this method, however, is that individual crystals tend to fall, or be plucked, out of the thin sections during preparation, leaving characteristically shaped holes (Dieppe & Calvert, 1983). This problem is exaccerbated if large crystal aggregates are present since insufficient tissue remains to produce a viable section.

Until recently there have only been limited reports of the use of scanning electron microscopy (SEM) to study crystals *in situ* in the articular cartilage (Reginato *et al*, 1974, Cameron *et al*, 1975, Nagahashi, 1979, Ali *et al*, 1983, Rees *et al*, 1986, and Ishikawa *et al*, 1989). SEM has several advantages compared to optical microscopy. These include higher magnifications (as a result of enhanced resolution) and a greater depth of field. This has particular relevance to the investigation of individual crystals *in situ*, which, because of their small size (1-20μm), are difficult to observe with the optical microscope. In addition a three-dimensional image of the crystals is obtained in the SEM, compared to a two-dimensional silhouette observed in the optical microscope. Energy dispersive analysis (EDA) can be used in conjunction with many

scanning electron microscopes to help identify the chemical composition of the crystals.

Descriptions of crystal aggregates present in the articular cartilage are varied. Dieppe (1978) summarised the most characteristic finding as rounded deposits of crystals in a granular matrix in the mid-zone, often in a line along the cartilage, resulting in a linear shadow on the x-ray picture. Reginato *et al* (1974), Cameron *et al* (1975), Linden & Telhag (1977) and Ishikawa (1989) descibed the crystal deposits as tophus-like, with several workers describing them as round, spherical or circular (Reginato *et al*, 1974, Cameron *et al*, 1975, Schumacher, 1976, Nagahashi, 1979). Only Ohira (1986) *et al* specified the size of the crystal deposits observed, being 1-1.5mm in diameter. Ali *et al* (1983) described large islands of crystals but did not specify a size and Linden & Telhag (1977) described crystal deposits ranging from a fine dust to large millimetre "pseudotophy".

Most reports described the crystal deposits as being distinctly outlined or well-demarcated (Bjelle, 1972, Schumacher, 1976, Nagahashi, 1979, and Mitrovic, 1983) Bjelle (1972) described dense calcifications lying close together but always "delineated" from one another. A thin zone surrounding the calcifications was observed that exhibited a higher level of stainability than the surrounding matrix with all dyes. It was concluded that this corresponded to an area of increased dry mass

concentration, possibly indicating the presence of a membrane. This zone was associated with peripheral crystals which were layered parallel to the border. He also observed that sometimes crystals at the periphery of the aggregate were more scattered with a radiating orientation. In this case no thin adjacent zone surrounded the crystal aggregate.

Most crystal deposits were reported to be located in the mid-zone (Bundens *et al*, 1965, Bjelle & Sundstrom, 1969, Bjelle, 1972, Reginato *et al*, 1974, and Schumacher, 1976). Some workers described seeing deposits in the apparent superficial layer after possible thinning of the cartilage or associated with fibrillation of the articular surface (Reginato *et al*, 1974, Schumacher, 1976, Linden & Telhag, 1977, Nagahashi, 1979, and Mitrovic, 1983). There was one report of crystals occuring in the deep zone (Bjelle, 1972). McCarty (1977) summarised the possible sites of CPPD deposits as: (i) perilacunar, (ii) large tophus-like masses within the cartilage layer, (iii) along clefts in degenerated cartilage, (iv) scattered in cartilage matrix of normal appearance, and (v) in the synovium.

Cartilage damage has often been reported in association with the crystal deposits (Reginato *et al*, 1974, Linden & Telhag, 1977, Schumacher, 1976, Mitrovic, 1983, Ohira *et al*, 1986, and Ishikawa, 1989), but several workers reported the presence of crystal deposits without any apparent damage to the cartilage (Bundens *et al*, 1965,

Currey *et al*, 1966, Lagier & Baud, 1968, Bjelle, 1972, and Mitrovic, 1983).

Reports in the literature of the distribution and morphology of large crystal aggregates were diverse, with few describing in detail any specific association of such aggregates with cartilage damage. An investigation was therefore undertaken with the SEM in order to provide detailed qualitative information with respect to large crystal aggregates and their association with any visible cartilage damage.

5.3 MATERIALS AND METHODS

In this study scanning electron microscopy (SEM) was used, in conjunction with freeze-fracturing and freeze-drying preparation techniques, to characterise the morphology and distribution of large crystal aggregates *in situ* in human post-mortem articular cartilage. This method resulted in minimal disruption of the surrounding tissue and circumvented the problems discussed above with preparing crystal-containing cartilage for optical microscopy. Energy dispersive analysis (EDA) (in conjunction with the SEM) (Ozawa & Yamamoto, 1983) and x-ray diffraction (XRD) (as described in section 3.4.3) were used to analyse the chemical composition of the crystals.

Samples of articular cartilage were taken *post mortem* from one patella and three femoral condyles of three

human knee joints. The anatomical sites of the samples taken, as characterised by Clift *et al* (1989), are given in table 5.1.

Specimen no.	Sex	Age (yrs)	Anatomical position	Fibrill-ation grade	Crystal type
23-1	F	80	P	S	CPPD
23-3	F	80	MC	M	CPPD
29-2	F	77	TC	S	CPPD
35-2	M	68	TC	S	MSU

Table 5.1 Specimen details. Anatomical position: P = patella, MC = middle of condyle, TC = top of condyle. Fibrillation: S = severe, M = mild, (Clift *et al*, 1989).

The cadavers were of either sex with an average age of 76 years. The physical appearance of the surface of the cartilage was also graded according to Clift *et al* (1989) by means of a modification of the criteria originally proposed by Sokoloff (1969). These are also given for each specimen in table 5.1.

Following fixation with formal saline, 5mm thick sections of cartilage and subchondral bone were radiographed in order to identify any crystal deposits (Watt, 1983). Slivers of articular cartilage taken from these crystal-rich areas were then frozen in liquid nitrogen, fractured and then freeze-dried in an Edwards-

Pearse Tissue Dryer at -60^{o}C for 12 hours to minimise tissue distortion. The specimens were coated with gold or carbon prior to examination in one of two scanning electron microscopes, a JEOL JSM-35C or a JEOL-T330, with accelerating voltages of between 10 and 20kV. EDA, by means of a Link Systems AN10000 analyser attached to the JSM-35C microscope, was used to analyse the composition of some of the crystal deposits.

Large crystal aggregates were also dissected with a scalpel from specimens of equine and human articular cartilage under a Leitz stereoscopic optical microscope. XRD of two crystal aggregates isolated from equine cartilage and one from human cartilage was used to confirm the composition of the individual crystals. Crystals from equine cartilage were also extracted from the cartilage by the technique of Swan *et al* (1992). The cartilage samples were incubated with papain until the cartilage had disintegrated. The suspension was then centrifuged and the pellet resuspended in distilled water and briefly washed in hypochlorite solution to remove any residual organic debris from the crystals. These extracted crystals were also analysed by XRD.

5.4 RESULTS AND DISCUSSION

Large aggregates of crystals were observed in all four of the human specimens. All but one of these aggregates consisted of individual crystals of triclinic and

monoclinic calcium pyrophosphate dihydrate (CPPD). However, an aggregate of monosodium urate monohydrate (MSU) crystals was also identified, by EDA, within the mid-zone of the articular cartilage of one of the femoral condyles, (Hayes *et al*, 1992).

Aggregates of octacalcium phosphate and a mixture of monoclinic and triclinic calcium pyrophosphate dihydrate crystals were identified by XRD in the equine articular cartilage.

5.4.1 Monosodium Urate Crystal Aggregate in Articular Cartilage

Since MSU crystals are not radio-opaque their presence in articular cartilage cannot be identified by the use of x-rays. The observation of an aggregate of MSU crystals (identified by EDA) within the cartilage layer was therefore unexpected. It was also surprising to find such an aggregate deep within the midzone as previous reports of MSU crystals present in the articular cartilage quote the superficial zone as the predominant location of these crystals (Sokoloff, 1957, Katz, 1975, and Dieppe *et al*, 1986). The individual MSU crystals within the crystal aggregate were a distinctive bow-shape (figure 5.1). This was in contrast to the needle-shaped crystals usually observed in synovial fluid (Paul *et al*, 1983).

MSU crystals grown by McGill *et al* (1989) *in vitro* in the presence of serum, synovial fluid and the components thereof closely resembled the morphology of the MSU crystals observed *in vivo* in the articular cartilage, whereas those grown without the presence of any biological components were needle-like. McGill *et al* (1989) concluded from their experiments that crystal formation was influenced by the biological environment present at the time of crystal formation. Addadi and Weiner (1985) also stated that owing to the uniform size, oriented crystallographic axes and well-defined crystal habits, crystals grown in biological environments must form under well-controlled conditions. It is interesting to note that *in vivo* two different morphologies of MSU crystal can exist in the articular cartilage and the synovial fluid, respectively. This would suggest that the physical medium in which such crystals are grown may also be an important factor in the determination of the formation of the crystals.

5.4.2 Calcium Pyrophosphate Dihydrate Crystal Aggregates in Articular Cartilage

Two types of CPPD deposit were observed in human articular cartilage. The first consisted of dense aggregates of crystals up to 700μm in diameter clearly separated from one another by a layer of tissue of variable thickness. A part of one such aggregate is shown in figure 5.2. Most of the densely-packed crystal

aggregates had a sharp demarcation between the edge of the aggregate (figure 5.2) and the adjacent cartilage matrix with no evidence of any organic tissue within the aggregate, (figure 5.3). However, at the edge of some aggregates there was some evidence of organic tissue amongst randomly-distributed crystals (figure 5.4). These densely-packed aggregates were regular in shape i.e. approximately spherical or ovoid, and they ranged in diameter from 50-700μm, although most were in the range of 50-100μm. The larger aggregates (>100μm) were usually observed in isolation deep within the cartilage layer and not confined to the midzone as previously reported (Reginato *et al*, 1974, Genant, 1976, Resnick *et al*, 1977, and Rees *et al*, 1986). Clusters of smaller aggregates (50-100μm) were observed near the articular surface. Each aggregate within these clusters was clearly delineated as described by Bjelle (1972). Such aggregates were always associated with fibrillation of the cartilage surface (figure 5.5).

Two large crystal aggregates dissected from equine articular cartilage also provided evidence of a well-defined interface between the cartilage matrix and the crystal aggregate. The crystals within one of these aggregates were loosely bound and the aggregate crumbled easily when pressure was applied with the tip of a scalpel blade although some crystals remained stuck to the capsule-like cartilage interface. However, crystals within the other aggregate were more tightly bound and

the aggregate did not crumble easily, requiring cutting from the cartilage.

In contrast to the densely-packed crystal aggregates groups of crystals were observed with a more random distribution and a less well-defined interface with the surrounding organic matrix, as illustrated in figure 5.6. These more loosely arranged crystal aggregates were interspersed with organic tissue and were less than 50μm in diameter. In some cases holes were observed between the crystals and the tissue. These crystal groupings were also closely associated with surface fibrillation.

Individual crystals within both types of aggregate were rods or rhomboids and varied in size from 0.5-2.0μm in width and 2-10μm in length. XRD (see appendix, table A7) and EDA (figure 5.7) confirmed that the individual crystals in both types of deposit were mixtures of monoclinic and triclinic CPPD.

5.4.3 The Association of the Crystal Aggregates With Cartilage Damage

Many of the small densely packed crystal aggregates (50-100μm in diameter) were associated with vertical cracks, or fibrillations, running deep into the articular cartilage. The more regularly-shaped aggregates were often associated with cracks which had either partially propagated up to or around the aggregate (figure 5.8). However, there were many irregularly-shaped crystal

deposits which were also associated with deep fibrillations of the cartilage. Some of these were positioned in gaps apparently left by a propagating crack (figure 5.9); other deposits were distributed along the edge of a crack (figure 5.10).

Results also indicated that large densely-packed crystal aggregates (>100μm in diameter) occuring in the lower half of the cartilage layer were not associated with any visible cartilage damage. This supported evidence provided by Currey *et al* (1966), Lagier & Baud (1968), Mitrovic (1983) and McCarty (1986), that crystal deposits can exist within the cartilage layer without any apparent damage to the cartilage. It thus appears that such large aggregates deep in the cartilage layer may not initiate mechanically-induced damage of the articular cartilage as previously expected, (Dieppe *et al*, 1983). Table 5.2 summarises the type of crystal deposit observed and its association with any cartilage damage. Figure 5.11 gives a schematic depiction of the results summarised in table 5.2.

Aggregate diameter (μm)	Depth in cartilage	Morphology of aggregate	Association with damage
<50	just below articular surface	loosely-packed	fibrillated articular surface
50-100	below art. surface - possibly in mid-zone originally	regular, densely-packed	fibrillated articular surface,
50-100	below surface - apparently in mid-zone	irregular, densely packed	follow line of cracks, "wedged in cracks"
<700	deep, calcified zone	regular, densely-packed	no visible damage to cartilage

Table 5.2 Summary of the size, location and morphology of crystal aggregates observed in articular cartilage and their association with visible cartilage damage.

It is feasible that the clusters of aggregates observed near the articular surface, may have originally grown in the mid-zone and as a consequence of degeneration of the cartilage and a subsequent reduction in its apparent thickness they appeared near the articular surface (Reginato *et al*, 1974, and Schumacher, 1976). In support of this argument such aggregates were always associated with fibrillation and visible damage of the articular cartilage in the form of deep vertical clefts. Also associated with a fibrillated articular surface were loosely packed crystal groups interspersed with an open mesh of organic tissue. It is suggested that these could

be the remains of an aggregate from which the individual crystals have begun to disperse into the synovial fluid. Such evidence would support the theory of Bennet *et al* (1976), Doherty & Dieppe (1981) and McCarty (1986), and the experimental evidence provided by Ohira *et al* (1986), who postulated that shedding of crystals into the joint space could be one mechanism by which crystals are released into the synovial fluid, thus initiating the painful episodes of acute "pseudogout".

The SEM results showed a close association between crystal deposits and damage to the cartilage, but it was not possible to tell whether the crystal aggregates were the primary cause of the cartilage damage.

5.4.4 Characterisation of Crystals in Equine Articular Cartilage

The presence of crystals in equine articular cartilage has not been reported previously. During dissection of 146 equine fetlock joints (73 animals) opaque white deposits were observed beneath the articular surface in 4 joints. Two aggregates from one particular joint were dissected from the cartilage with a scalpel (as already described in section 5.3). XRD confirmed that the crystals from both these aggregates were probably octacalcium phosphate (see appendix, table A8), although the XRD pattern was not very clear, exhibiting significant evidence of an amorphous phase. Mrs. A.

Swan, of the Rheumatology Unit, Bristol Royal Infirmary, carried out the crystal extraction procedure (Swan *et al*, 1992) on cartilage from three other such joints. Visible quantities of crystals were extracted only from one of these joints (figure 5.12). Polarised light microscopy and XRD confirmed that these crystals were a mixture of triclinic and monoclinic CPPD, as seen in human articular cartilage (see appendix, table A9).

5.5 CONCLUSIONS

Results from this work confirmed previous reports of crystal deposits found in the articular cartilage (Bjelle, 1972, Cameron *et al*, 1975, Watt, 1983, Mitrovic, 1983, and Ali *et al*, 1983), but with several unique observations. These included: (i) an aggregate of bow-shaped MSU crystals (rather than needles) within the midzone of the cartilage layer, rather than the superficial zone, (ii) large aggregates of CPPD crystals distributed throughout the thickness of the articular cartilage and not just confined to the midzone, and (iii) two distinct types of CPPD aggregate identified with either densely packed, or loosely arranged, crystals.

Observations of large crystal aggregates in isolation deep within undamaged cartilage indicated that the presence of such aggregates need not necessarily be detrimental to the cartilage as a whole. This conclusion

is supported by clinical observations. However, when a high concentration of crystal aggregates was present close to the articular surface this was always associated with fibrillation of that surface and cracks throughout much of the thickness of the cartilage. This study, therefore, provided evidence that suggested that crystal aggregates were associated with cracks within the cartilage, but no conclusions could be drawn with respect to whether the aggregates preceded the cracks or vice versa.

Characterisation of crystals in human articular cartilage by XRD confirmed the presence of a mixture of monoclinic and triclinic calcium pyrophosphate dihydrate. Characterisation of crystal aggregates observed in equine fetlock articular cartilage, also by XRD, confirmed that crystals of octacalcium phosphate and a mixture of triclinic and monoclinic CPPD were observed in two separate joints.

This investigation has provided detailed information with respect to the morphology and distribution of large crystal aggregates within the articular cartilage. These data can be used with analysis techniques such as finite elements for modelling stresses induced in such a material in further studies. Results from such analysis would help to clarify the hypothesis that the onset of physical degradation of articular cartilage is mediated, in part, by the presence of large crystal deposits within the cartilage layer.

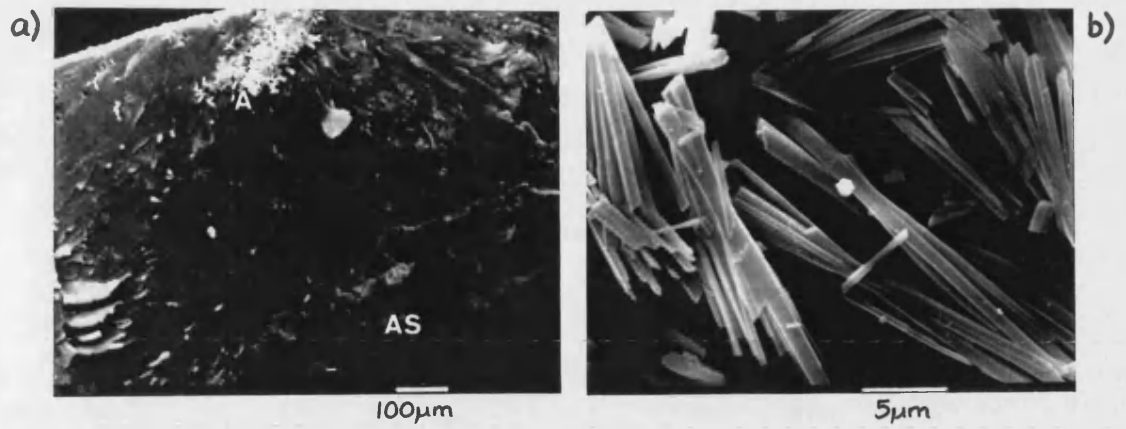


Fig. 5.1 SEMs of a) an aggregate (A) of MSU crystals within the midzone of the articular cartilage, and b) the individual bow-shaped MSU crystals. (AS = articular surface).

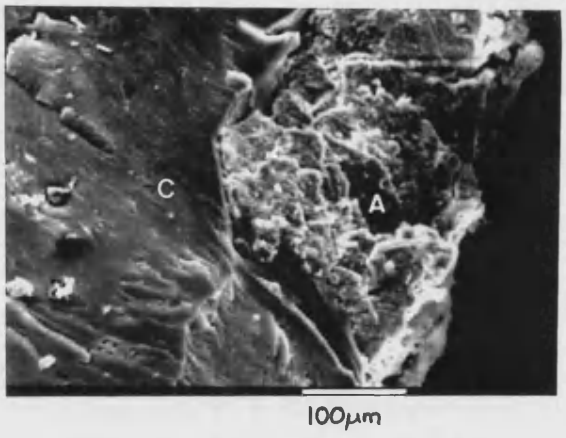


Fig. 5.2 SEM of part of a densely packed aggregate (A) of CPPD crystals. (C= cartilage).

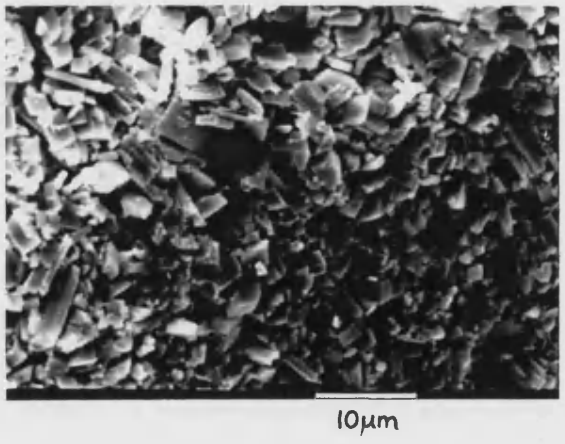


Fig. 5.3 SEM of the individual crystals within a densely packed aggregate illustrating the lack of organic tissue.

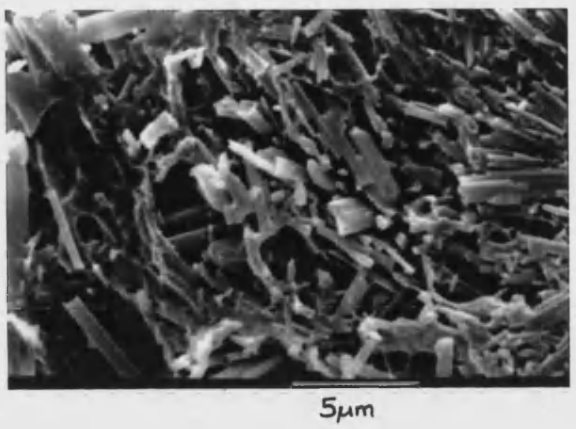


Fig. 5.4 SEM of the edge of a densely packed aggregate showing individual crystals interspersed with organic tissue.

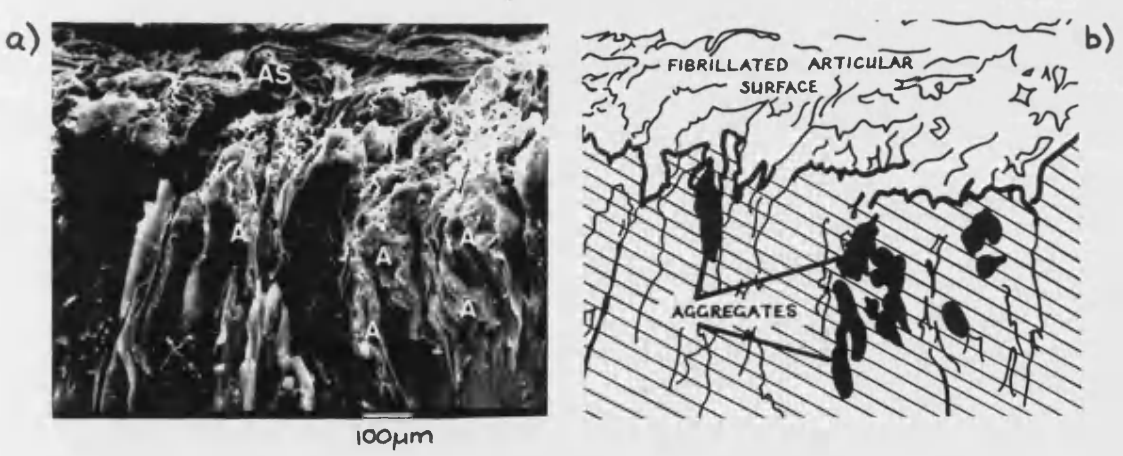


Fig. 5.5 SEM (a) and a schematic diagram (b) of clusters of densely packed aggregates (A) associated with a fibrillated articular surface (AS).

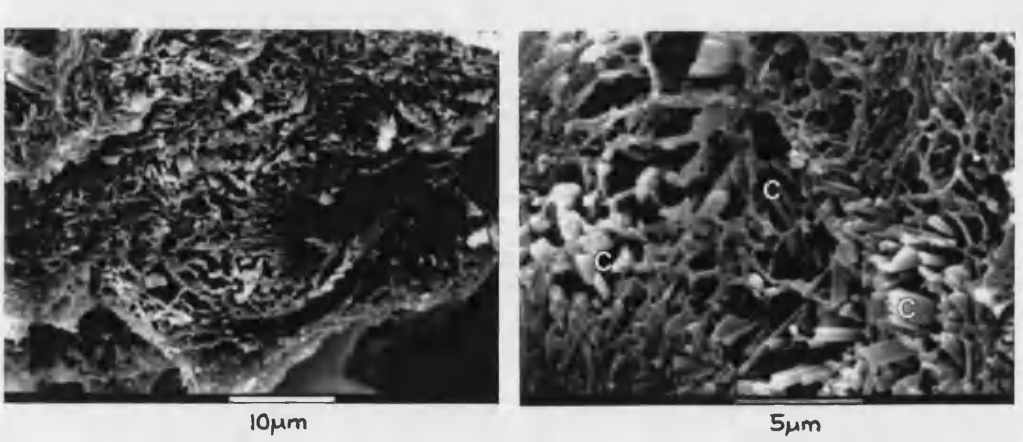


Fig. 5.6 SEMs of a loosely packed crystal aggregate. (C = crystals).

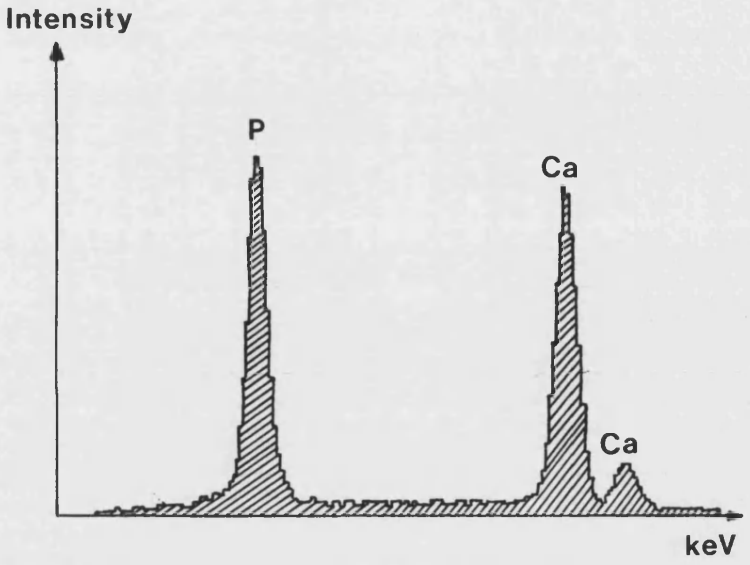


Fig. 5.7 EDA of a typical crystal aggregate.

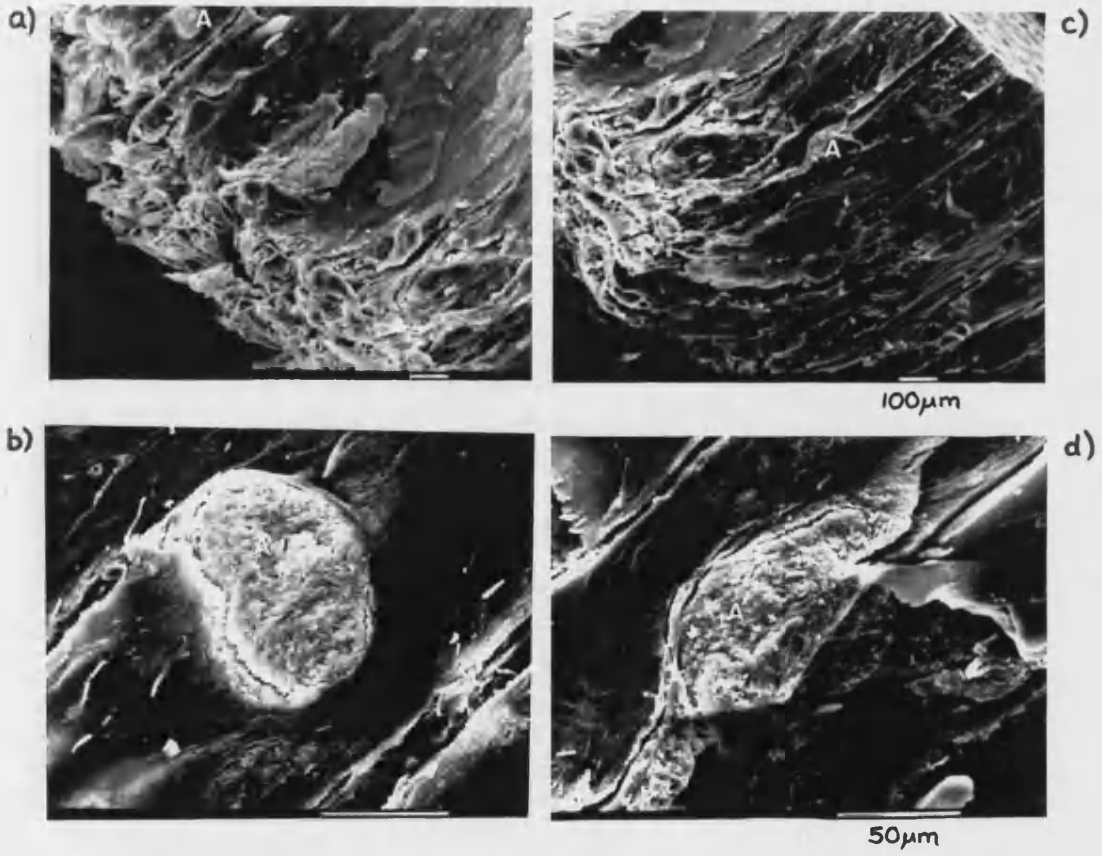


Fig. 5.8 a) and b) SEMs of a crack propagating up to a densely packed aggregate (A), and c) and d) SEMs of a crack having propagated around a densely packed aggregate (A) (low and high magnifications respectively).

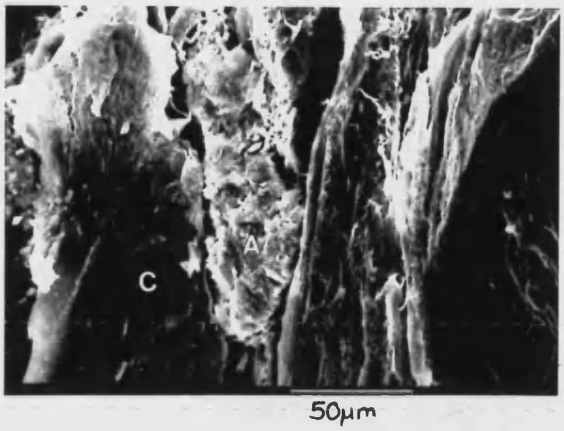


Fig. 5.9 SEM of a densely packed aggregate (A) positioned in a gap apparently left by a propagating crack. (C = cartilage).

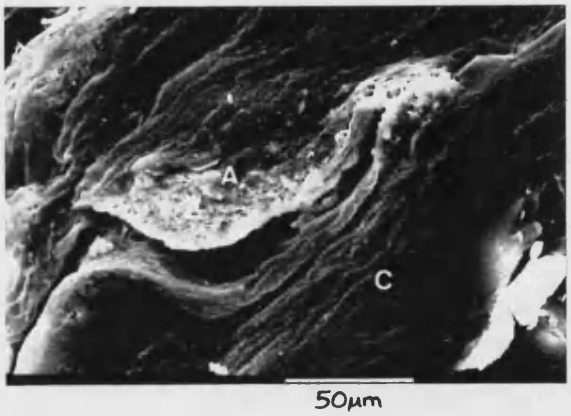


Fig. 5.10 SEM of a crystal deposit (A) distributed along the edge of a crack. (C = cartilage).

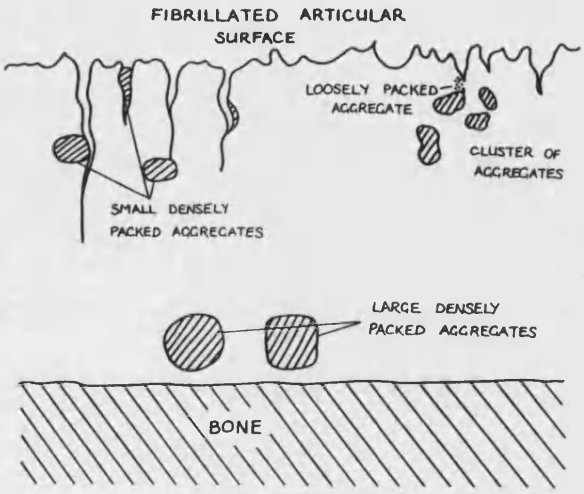


Fig. 5.11 A schematic diagram illustrating the relative positions within the cartilage layer of the different types of crystal aggregate.

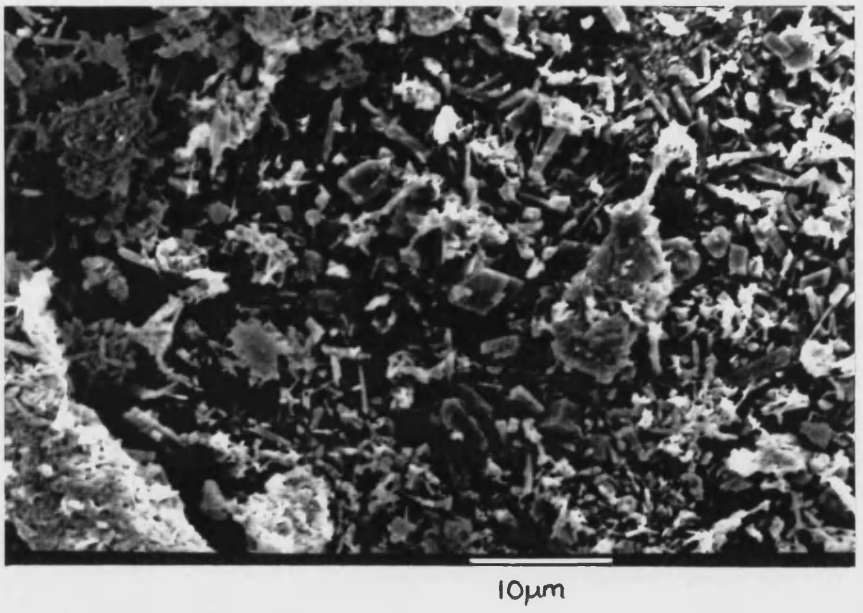


Fig. 5.12 Monoclinic and triclinic CPPD crystals extracted from equine articular cartilage.

6. CONCLUSIONS AND FURTHER WORK

The original aims of this research, as stated in the introduction (chapter 1) were: (i) to investigate whether crystals in a lubricant could cause damage to articular cartilage *in vitro*, and (ii) to investigate any association between crystal deposits within the cartilage and visible cartilage damage. Measurements of the frictional and wear response of articular cartilage with and without crystals on the surface or in the lubricant were carried out in order to investigate the potential damage that crystals could cause to the articular surface. Transverse sections of human articular cartilage were studied by scanning electron microscopy in order to investigate any association between crystal deposits and cartilage damage.

6.1 The Frictional Response of Articular Cartilage With and Without Crystals

Investigations of the frictional response of articular cartilage with and without surface crystals were limited owing to problems encountered with the apparatus. However, results indicated that the coefficient of friction varied with crystal type. It was suggested that crystal morphology was an important factor. Needle-like HA crystals on the articular surface increased the coefficient of friction whereas work completed by Hailey

(1989) showed that rhomboid CPPD crystals on the articular surface reduced the coefficient of friction. It had been assumed throughout these experiments that a direct correlation existed between the coefficient of friction of articular cartilage and the potential for damage of the articular surface. No such relationship, however, has ever been established. Further work, therefore, concentrated upon obtaining a direct measure of wear, or damage, of the articular cartilage with crystals present in a lubricant.

6.2 The Wear Response of Articular Cartilage With and Without Crystals

A successful technique was established to measure the wear of articular cartilage *in vitro* by analysing the concentration of cartilage debris in the lubricant. The concentration of inorganic sulphate (originating from the cartilage proteoglycans) was analysed by ion chromatography. However, owing to the variable concentration of sulphated GAGs with depth through the cartilage it proved difficult to quantify accurately the amount of cartilage removed. It is suggested that any further work should include analysis of the hydroxyproline content (derived from the collagen) in the lubricant as this is reported to be constant throughout the depth of the cartilage, (Lipshitz & Glimcher, 1979). A direct correlation could then be established between the concentration of cartilage

debris in the lubricant and the amount of cartilage removed.

Results obtained by ion chromatography indicated that the wear response of normal, undamaged articular cartilage was dependent on the load and also on the nominal contact stress applied. The presence of crystals in the lubricant significantly increased the wear of the cartilage, with twice the inorganic sulphate concentration measured in these lubricants as for those not containing crystals. However, measurement of the vertical displacement of the cartilage during the wear test, together with data obtained for the contact areas before and after the wear tests, indicated that the overall volume of material removed was the same for all cases (with and without crystals). Further analysis of the wear specimens with SEM suggested that the cartilage surrounding the contact area, but still immersed in the lubricant, was also significantly damaged. It was suggested that this could have resulted in the higher inorganic sulphate concentration measured in the lubricants containing crystals. SEM also provided evidence to suggest that the type of wear damage incurred by the cartilage was influenced by the crystal type, even though the concentration of cartilage debris (measured by ion chromatography) was the same for all crystal types.

It is anticipated that any further work in this area would include the simultaneous measurement of friction

and wear in order to clarify the hypothesis that a high coefficient of friction is directly correlated with a high potential for damage, or wear. Simultaneous measurement of the frictional properties during a wear test would also help to establish the type of wear mechanism taking place.

6.3 The Wear Response of Articular Cartilage Scratched *In Vivo*

Cartilage that had been scratched *in vivo* generally showed no significant difference in its wear response compared to normal, undamaged cartilage, although there was some evidence that cartilage with scratch marks easily visible by eye released greater concentrations of sulphated GAGs into the lubricant than normal, undamaged cartilage or badly scratched cartilage. There was, therefore, no clear evidence to suggest that the wear response of the cartilage had been compromised by the scratch marks. SEM indicated that the clear parallel scratch marks observed by eye consisted of a finely fibrillated surface with loose fibrils perpendicular to the length of the scratch mark. It is envisaged that any future work would carry out analysis of the stored synovial fluids corresponding to each specimen for any particulate matter that could have produced these scratch marks. Suitable extraction methods (developed by Moradi, 1991, and Swan, 1992) followed by polarised light microscopy, electron microscopy in conjunction

with energy dispersive analysis and x-ray diffraction could be used to analyse the nature of the particles. Alternatively, ferrography (Evans *et al*, 1981) followed by microscopy could be used.

6.4 The Nature of the Relative Bearing Surfaces

A major criticism of this work has been that the hard opposing surface to the cartilage (the stainless steel plate) bears little resemblance to the *in vivo* situation of two soft cartilage surfaces in contact. The mechanisms of damage would, therefore, be different from those existing *in vivo*. A further understanding of potential *in vivo* conditions might be obtained *in vitro* by using a soft bearing material, such as the polyurethanes used by Unsworth (1991), instead of the relatively hard stainless steel.

6.5 The Association Between Crystal Aggregates and Cartilage Damage

SEM observations of crystal aggregates *in situ* in human articular cartilage provided evidence of (i) individual crystal aggregates in or above the mid-zone often associated with cracks in the cartilage and (ii) high concentrations of crystal aggregates near the articular surface always associated with fibrillation of that surface and cracks throughout the thickness of the cartilage. However, large isolated crystal aggregates

were observed deep within undamaged cartilage. Information provided by this study will be used in conjunction with finite element analysis and photoelasticity to model stresses within cartilage containing such aggregates. It is anticipated that such data will help clarify the hypothesis postulated by Dieppe and his colleagues that the presence of crystal deposits in the cartilage could alter its compliance thus increasing the potential for cartilage damage.

6.6 Summary

In conclusion, this thesis has provided long-awaited evidence to support the hypothesis that crystals can cause mechanical damage of articular cartilage *in vitro* by surface abrasion, in particular, and that the morphology of the crystal influences the type of damage caused.

ACKNOWLEDGEMENTS

An important contribution to the success of this program of research was the help and advice provided by many colleagues. I would like to thank the members of the Crystal Research Group, based at the Department of Rheumatology, Bristol Royal Infirmary, for their friendly support and their patient explanations of anything biological. In particular, I would like to thank Dr. Peter Shellis for willingly supplying copious quantities of crystals and for the use of the ion chromatograph, and I would like to thank Mrs. Margaret Marshall for her reliable technical assistance when obtaining the ion chromatography data. Mrs. Angela Swan also deserves thanks for supplying some of the synthetic crystals and for her help in extracting the biological crystals from the cartilage. I would also like to thank Dr. Brigid Heywood for her unfailing enthusiasm and advice with respect to anything involving crystals.

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Finally, I would like to thank my two supervisors, Prof. Paul Dieppe and Prof. Bryan Harris. I would like to thank Paul for all his words of wisdom with respect to arthritic diseases and the relevance, or not, of physiological representation. I hope that this thesis will help to answer the question which he asked at my first project meeting: "Can crystals damage articular

cartilage?" I would like to thank Bryan for his continued support of the work and his availability for interesting and enlightening discussions (well, sometimes anyway!). I would also like to thank him for creating an atmosphere in which I found it easy to learn. I thoroughly enjoyed working with him.

APPENDIX

d-spacing HA13	Intensity HA13	Index card 9-432 d-spacing HA	Index card 9-432 I/I_1 HA	
8.42	f	8.17	12	
7.95	f			
		5.26	6	
		4.72	4	
4.13	f	4.07	10	
3.87	f	3.88	10	
3.48	m	3.51	2	
3.39	m	3.44	40	
3.13	f	3.17	12	
3.04	f	3.08	18	
2.84	m	3.814	100	
2.80	s	2.778	60	
2.74	s	2.720	60	
2.69	s			
2.66	f			
2.61	m	2.630	25	
2.51	f	2.528	6	
2.28	m	2.296	8	
2.25	m	2.262	20	
		2.228	2	
2.14	f	2.148	10	
		2.134	4	
2.06	f	2.065	8	
		2.040	2	
1.99	f	2.000	6	
1.95	m	1.943	30	
1.93	s			
1.89	m	1.890	16	
		1.871	6	
1.84	s	1.841	40	
		1.806	20	
1.79	m	1.780	12	
1.77	m			
1.75	m	1.754	16	
1.71	s	1.722	20	
		1.684	4	
1.64	f	1.644	10	
1.61	f	1.611	8	

Table A1. X-ray diffraction data for synthetic hydroxyapatite crystals used in the friction experiments. (Intensity: s=strong, m=medium, f=faint.)

d-spacing	Intensity	Index card 28-233 d-spacing	I/I_1	Index card 22-536 d-spacing	I/I_1
CPPD		t-CPPD		m-CPPD	
8.04	s	8.01	100		
7.15	s			7.37	100
6.88	s	6.95	100		
				6.08	10
		5.48	10		
				5.27	10
		5.20	10		
4.80	f				
4.67	s			4.62	90
		4.47	10		
4.33	f				
4.09	f				
4.00	f	3.98	20		
3.85	m				
		3.73	5	3.74	40
3.65	m				
3.44	m	3.42	60	3.43	10
3.27	f				
3.21	s	3.21	80	3.22	80
3.11	s	3.10	80		
3.05	m	3.04	60	3.03	95
2.95	m	2.95	40		
2.83	f			2.86	10
2.77	f	2.76	40	2.77	40
2.66	m	2.65	60		
2.61	f			2.63	70
2.50	f	2.50	20	2.56	60
2.46	f			2.41	40
2.32	m	2.32	10	2.30	60
2.25	s	2.23	40	2.20	10
2.12	m			2.10	40
2.07	m	2.09	40		
2.04	m				
2.00	m	2.00	20	2.00	10
1.90	m	1.89	20	1.89	60
1.85	f				
1.83	f			1.83	40
1.75	m	1.73	40	1.76	40
1.71	m			1.69	40

Table A2. X-ray diffraction data for synthetic calcium pyrophosphate dihydrate crystals used in the friction tests and the pilot wear experiments. (Intensity: s=strong, m=medium, f=faint.)

d-spacing	Intensity	Index card 11-293 d-spacing	I/I_1	
DCPD		**Brushite**		
7.61	s	7.62	100	
4.23	s	4.27	2	
		3.80	30	
3.05	s	3.06	8	
2.93	s	2.93	1	
2.86	m	2.86	<1	
2.81	f	2.80	<1	
		2.67	1	
2.62	s	2.63	1	
2.56	f	2.53	6	
2.42	s	2.47	<1	
		2.27	1	
2.16	m	2.17	1	
		2.15	<1	
		2.10	<1	
2.09	m	2.09	<1	
2.03	f	2.03	<1	
2.00	m	2.01	2	
1.97	m	1.98	<1	
		1.94	<1	
1.90	m	1.90	10	
1.86	m	1.86	1	
1.82	s	1.82	<1	
1.80	f	1.80	<1	
		1.78	<1	
1.71	m	1.71	<1	
1.65	m	1.63	<1	
		1.62	<1	
1.61	f	1.61	<1	
		1.59	<1	
1.55	s	1.55	<1	
		1.53	<1	
1.52	m	1.52	<1	
1.45	s	1.46	<1	
1.44	m			
1.41	f			
1.40	f			
1.38	f			
1.37	f	1.37	<1	
1.34	m	1.34	<1	
1.32	f	1.33	<1	
1.30	m			
1.28	f	1.27	1	

Table A3. X-ray diffraction data for synthetic brushite crystals used in the pilot wear experiments. (Intensity: s=strong, m=medium, f=faint.)

d-spacing o-CPPT	Intensity o-CPPT	Index card 22-535 d-spacing o-CPPT	I/I_1	
10.7	s	11.4	100	
6.33	m			
		5.95	40	
5.72	m			
5.29	f	5.37	10	
		4.99	10	
4.73	m	4.70	10	
4.28	f	4.37	40	
		3.75	40	
3.51	s			
3.30	s	3.37	60	
3.17	s	3.18	20	
3.12	f	3.05	40	
2.91	s	2.94	80	
2.82	s	2.83	20	
2.71	m	2.65	20	
2.47	m	2.56	20	
2.45	f			
2.35	f	2.35	10	
2.30	f			
2.23	m	2.26	10	
2.18	f	2.16	40	
2.10	m	2.12	40	
2.05	m	2.04	20	
2.01	f			
1.92	m	1.93	20	
1.88	f	1.84	20	
1.79	m	1.78	0	
1.76	f			
1.74	m	1.73	20	
1.71	f			
1.65	f			
1.63	m	1.61	10	
1.59	f			
1.57	f	1.57	20	
1.54	f	1.53	10	
1.48	f	1.39	10	

Table A4. X-ray diffraction data for synthetic orthorhombic calcium pyrophosphate tetrahydrate crystals used in the wear experiments. (Intensity: s=strong, m=medium, f=faint.)

d-spacing m-CPPD	Intensity m-CPPD	Index card 22-536 d-spacing m-CPPD	I/I_1	
7.37	s	7.37	100	
6.00	f	6.08	10	
5.29	f	5.27	10	
4.61	s	4.62	90	
3.71	m	3.74	40	
3.43	m	3.43	10	
3.24	s	3.22	80	
3.03	s	3.03	95	
2.95	f	2.86	10	
2.78	m	2.77	40	
2.66	s	2.63	70	
2.56	m	2.56	60	
2.41	m	2.41	40	
2.30	m	2.30	60	
2.25	f			
2.20	f	2.20	10	
2.13	m	2.10	40	
2.01	m	2.00	10	
1.90	m	1.89	60	
1.84	m	1.83	40	
1.77	m	1.76	40	
1.74	f			
1.69	f	1.69	40	

Table A5. X-ray diffraction data for monoclinic calcium pyrophosphate crystals used in the wear experiments. (Intensity: s=strong, m=medium, f=faint.)

d-spacing HA22	Inten-sity	Index card 9-432 d-spacing HA	I/I_1	
9.07	f			
8.23	m	8.17	12	
5.25	m	5.26	6	
4.73	f	4.72	4	
4.09	m	4.07	10	
3.81	f	3.88	10	
		3.51	2	
3.43	s	3.44	40	
3.17	m	3.17	12	
3.08	m	3.08	18	
2.82	s	2.814	100	
2.78	s	2.778	60	
2.71	s	2.720	60	
2.63	m	2.631	25	
2.53	f	2.528	6	
2.30	f	2.296	8	
2.25	s	2.262	20	
		2.228	2	
2.15	f	2.148	10	
		2.134	4	
2.06	f	2.065	8	
		2.040	2	
2.00	f	2.000	6	
1.94	m	1.943	30	
1.89	m	1.890	16	
		1.871	6	
1.84	m	1.841	40	
1.80	m	1.806	20	
1.78	m	1.780	12	
1.75	m	1.754	16	
1.72	m	1.722	20	
1.68	f	1.684	4	
1.64	f	1.644	10	
1.61	f	1.611	8	
1.58	f	1.587	4	
1.54	f	1.542	6	
1.53	f	1.530	6	
1.50	m	1.503	10	
1.47	m	1.474	12	
1.45	m	1.465	4	

Table A6. X-ray diffraction data for synthetic hydroxyapatite crystals used in the wear experiments. (Intensity: s=strong, m=medium, f=faint.)

d-spacing	Intensity	Index card 28-233 d-spacing	I/I_1	Index card 22-536 d-spacing	I/I_1
CPPD		t-CPPD		m-CPPD	
8.42	s	8.01	100		
7.78	s				
7.23	s			7.37	100
		6.95	100		
6.68	s				
				6.08	10
5.37	f	5.48	10		
		5.20	10	5.27	10
5.07	f				
				4.62	90
4.50	f	4.47	10		
4.11	f				
3.95	f	3.98	20		
3.71	f	3.73	5	3.74	40
3.53	f				
3.39	f	3.42	60	3.43	10
3.29	f				
3.19	s	3.21	80	3.22	80
3.08	s	3.10	80		
3.03	m	3.04	80	3.03	95
2.93	m	2.95	40		
2.82	f			2.86	10
2.74	m	2.76	40	2.77	40
2.64	m	2.65	60	2.63	70
2.56	f			2.56	60
2.49	f	2.50	20		
2.37	f			2.41	40
2.32	f	2.32	10		
2.28	f			2.30	60
2.24	m	2.23	40	2.20	10
2.10	m	2.09	20	2.10	40
2.06	f			2.00	10

Table A7. X-ray diffraction data for an aggregate of crystals dissected from human femoral articular cartilage. (Intensity: s=strong, m=medium, f=faint.)

d-spacing OCP	Intensity	Index card 26-1056 d-spacing OCP	I/I_1	
		18.7	300	
		9.36	45	
		9.05	40	
7.38	s			
		6.10	6	
		5.52	25	
		5.417	7	
		5.211	4	
		5.101	12	
		4.815	6	
		4.706	5	
		4.670	4	
		4.514	10	
		4.492	10	
		4.294	7	
		4.111	5	
		3.919	16	
		3.879	12	
		3.862	10	
		3.786	10	
		3.745	14	
		3.660	30	
		3.492	25	
3.43	m	3.441	50	
		3.424	60	
		3.378	18	
		3.311	20	
		3.278	18	
		3.209	25	
		3.180	25	
		3.132	10	
3.11	f	3.117	7	
		3.055	14	
		3.015	8	
		2.946	14	
		2.914	12	
		2.873	30	
		2.833	100	
2.81	s	2.820	95	
		2.779	45	
		2.745	35	
cont.				

Table A8a. X-ray diffraction data for a crystal aggregate dissected from equine fetlock articular cartilage. (Intensity: s=strong. m=medium, f=faint.)

d-spacing OCP	Intensity	Index card 26-1056 d-spacing OCP	I/I_1	
		2.707	25	
		2.671	50	
		2.637	35	
2.62	m	2.617	20	
		2.606	20	
		2.567	16	
		2.544	12	
		2.486	5	
		2.475	8	
		2.458	5	
		2.365	7	
		2.335	8	
		2.304	7	
2.27	m	2.271	5	
		2.265	6	
		2.258	7	
		2.215	16	
		2.158	5	
		2.136	7	
		2.106	9	
		2.088	7	
2.06	f	2.063	6	
		2.036	5	
		2.002	8	
		1.998	9	
		1.990	10	
		1.957	7	
1.94	m	1.948	17	
		1.936	18	
		1.929	11	
		1.914	11	
		1.897	10	
		1.891	10	
		1.848	20	
1.84	m	1.837	20	
		1.832	18	
		1.804	15	
1.76	f	1.745	8	
		1.743	8	
1.72	m			
1.50	f			
1.45	f			

Table A8b. X-ray diffraction data for a crystal aggregate dissected from equine fetlock articular cartilage. (Intensity: s=strong. m=medium, f=faint.)

d-spacing	Intensity	Index card 28-233 d-spacing	I/I_1	Index card 22-536 d-spacing	I/I_1
CPPD		t-CPPD		m-CPPD	
8.04	s	8.04	100		
7.45	s			7.37	100
6.94	s	6.95	100		
				6.08	10
		5.48	10		
5.29	f			5.27	10
		5.20	10		
4.61	m			4.62	90
		4.47	10		
4.13	s	3.98	20		
3.73	f	3.73	5	3.74	40
3.46	m	3.42	60	3.43	10
3.24	s	3.21	80	3.22	80
3.12	m	3.10	80		
3.05	m	3.04	60	3.03	95
2.97	f	2.95	40		
				2.86	10
2.79	s	2.76	40	2.77	40
2.66	m	2.65	60	2.63	70
2.58	f			2.56	60
2.51	f	2.50	20		
2.41	f			2.41	40
2.35	f	2.32	10		
2.30	f			2.30	60
2.25	m	2.23	40		
2.20	f			2.20	10
2.12	m			2.10	40
2.07	f	2.09	40		
2.00	m	2.00	20	2.00	10
1.94	f				
1.91	f	1.89	20	1.89	60
1.84	m			1.83	40
1.76	f			1.76	40
1.71	f	1.73	40	1.69	40

Table A9. X-ray diffraction data for crystals extracted from equine fetlock articular cartilage.

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